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EDITOR
JOHN MERLE COULTER

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ERRATA

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- P. 332, in table I, light intensities in foot candles should be multiplied by the
factor 0.282, owing to an error in calculation
- P. 450, last line, for (p. 287^f) read [p. 287(1)]
- P. 451, lines 11 and 12, for the material obtained this year read the material of
1923

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- P. 182, in legend of fig. 7, for *Chenopodium album* var. *anthelminticum* read
Chenopodium ambrosioides var. *anthelminticum*



THE BOTANICAL GAZETTE

September 1924

MORPHOLOGY AND PHYSIOLOGY OF THE AXIS IN CUCURBITACEAE¹

ROLAND HOLROYD

(WITH PLATES I-III AND FIVE FIGURES)

Introduction

From the time of HARTIG, a copious literature has developed on the origin and history of the internal phloem. The writer investigated the embryology and developmental history of *Cucumis Melo* var. *flexuosus* Naud., in which he showed that the internal phloem arises by continuation inward from the normal intrafascicular cambium of cells that become cambioid, and that eventually surround the xylem. This zone produces, on its inner face adjoining the pith, the first rudiments of an internal phloem. Through continued cambial activity, the masses of internal phloem are gradually enlarged to form the well known bicollateral bundles of the Cucurbitaceae. This internal phloem maintains its position throughout. Observation of certain interesting details during the progress of that work induced the writer to engage in the study of the more advanced conditions in the root, hypocotyl, and gradually maturing stem of this and other genera of the Cucurbitaceae.

The presence of internal phloem in stems was first discovered and described by HARTIG (6)¹ in *Cucurbita Pepo* in 1854. VON MOHL (23) announced its presence, the year following, in

¹ Thesis presented to the Graduate School of the University of Pennsylvania in partial fulfilment of requirements for the degree of Doctor of Philosophy.

Apocynaceae, Solanaceae, and Compositae. SCHREIBER (15) in 1865 announced its presence in Lythraceae, and RUSSOW (13) in a great many diverse families. In addition, VESQUE (21), PETERSEN (9), and SOLEREDER (16, 17) give us a fair knowledge of the extent of this feature in other families of the dicotyledons. As given by SOLEREDER, they include Vochysiaceae, Melastomaceae, Myrtaceae, Lythraceae, Onagraceae, Cucurbitaceae, Asclepiadaceae, Apocynaceae, Solanaceae, Gentianaceae, and Loganiaceae, as well as in most Convolvulaceae and in some genera of the Acanthaceae, Thymelaeaceae, Penaeaceae, Euphorbiaceae, Polygonaceae, and Campanulaceae. It will be noticed that this advanced developmental condition is rare in the primitive dicotyledons or Incompletae (Apetalae); is more frequent in the Apopetalae (Polypetalae); and most frequent, as well as most perfectly evolved, in the Sympetalae (Gamopetalae).

DEBARY (2), referring to the leaf trace bundles in the Cucurbitaceae, states that phloem areas "are frequently connected by means of a narrow band, fringing the lateral edge of the bundle, and containing some sieve tubes, so that in these cases the bundle, strictly speaking, belongs to the concentric type." Further, referring to the leaf traces of the Melastomaceae and other families, he states that "in many of these the inner phloem is so widely separated from the rest of the bundle that it may best be regarded as a distinct strand of sieve tubes." DEBARY originated the term "bicollateral."

GERARD (4) traced the course of the bundles from the root to stem in *Cucumis Melo* and *Cucurbita maxima*, and found that the phloem of the root gave off strands, some of which were continuous with the internal phloem of the stem. He concluded that the internal phloem is merely a part of the external that gets placed on the inner face of the bundle.

PETERSEN (9) described the bicollateral bundle as agreeing with the collateral, with the addition of an internal phloem area.

WEISS (24) determined that all the bundles in the Cucurbitaceae were leaf trace bundles. In the petioles, at the point where the leaf veins branch off from the main bundles, the internal phloem unites with the external, and the bundles are no longer bicollateral.

FISCHER (3), in tracing the transition from hypocotyl to root, found that the internal phloem gradually died out and ended blindly below. He states that the internal phloem passes to the outside of the primary xylem and forms a ring between this and the secondary wood, disappearing lower down, but long remaining distinguishable in the medullary rays.

HERAIL (7) stated that the Cucurbitaceae alone possess a true bicollateral bundle, because the development of all three parts of the bundle is synchronous.

POTTER (11) observed that the mode of increase in thickness in the stem of *Cephalandra indica* is the same as that of ordinary dicotyledons. It differs in that its bundles are disposed in two circles rather than in one. He subscribes to the view of DEBARY that these two concentric rings of bundles behave as a single ring which bends alternately outward and inward. In a later paper (12) he calls attention to the fact that while climbing cucurbits possess a sclerenchymatous ring of cortical tissue, the shrubby members of the family, sufficiently supported by a relatively greater amount of xylem, do not possess this feature. Further, in the shrubby forms, the need for additional phloem is met by the formation of metaphloem by the intrafascicular cambium, and thus the amount of internal phloem is not large.

LAMOUNETTE (8) found that the external phloem had developed extensively in the region between the "heel" and the first rootlets of the hypocotyl, while the pith parenchyma was giving rise to an internal phloem. No communication was observed between the two. The formation of the internal phloem was thus subsequent to that of the external. LAMOUNETTE concluded that the internal phloem development is abnormal, and that it is due to the activity of certain pith cells. He advocated the abandonment of the term "bicollateral" in view of this method of origin.

SCOTT and BREBNER (14) indicate the connection of the internal and external phloem areas. "In the transitional regions the strands of internal phloem successively pass out between the converging xylem bundles and one by one reach the strands of the external phloem, with which they fuse." The writer does not subscribe to this centrifugal behavior of the internal phloem. The authors

suggest that the internal phloem is of advantage in that it is phloem sheltered by the wood cylinder, that the tissues are more concentrated, and that communication with the pith is more easily established.

BARANETSKY (1) states that "the internal phloem bundles in the Cucurbitaceae, when provided with their own wood, represent, doubtless, independent vascular bundles." Their independence is demonstrated by their branching and by their passing from one normal bundle to another. He would regard the presence of internal phloem as an evolutionary development, and cites its presence in some of the Sympetalae in support of this idea.

PITARD (10) found in *Cucurbita Pepo* strands of phloem in the rays of the stem at the edge of the wood which are connected by branches with the internal phloem of the bundles.

TONDERA (20), investigating *Sicyos angulatus*, concluded that the internal phloem is derived without cambial action, and is placed directly in front of the protoxylem. He indicates the presence of seven outer and three inner bundles (instead of five outer and five inner), confounding two bundles which, as often happens, are of greatly reduced size as compared with the other three. He considers that the two smallest bundles, which he terms of the outer circle and which in reality are of the inner, consist of phloem without any xylem. His paper is concerned with tracing the relation and disposition of the vascular bundles in connection with the leaf supply.

VON FABER (22), studying *Cucurbita Pepo*, found that at the growing apex both external and internal cambiums appear on either side of the developing vascular bundle, before any visible differentiation of spiral or other wood elements can be traced. He favors the opinion of BARANETSKY (1) with regard to exotic Cucurbitaceae, where not only an internal cambium, but also an internal xylem arises on the inner face of the phloem and cambium (corresponding to the "obcollateral bundles" of the writer), so that an internal phloem represents a developing commencement of a complete bundle. He upholds the term "bicollateral," and regards the development of both phloem areas as being centripetal, that is, toward the protoxylem.

MISS THOMPSON (19), in a study of the anatomy of *Gelsemium sempervirens*, indicates the absence of any internal phloem in the

lower part of the hypocotyl and the root. Two of the internal phloem patches arise just below the cotyledonary node, the other two just below the node bearing the first pair of epicotylary leaves. She states that the internal phloem is an acquired characteristic of the plant, and that it has probably been developed to supplement the external phloem in the discharge of its functions.

WORSDELL (25), after an investigation of the anatomy of *Cucurbita*, *Acanthosicyos*, etc., concludes that "medullary phloem represents . . . a vestigial structure, the remnant of a former system of medullary vascular bundles in which the xylem has disappeared." This phenomenon has been retained solely because of its rôle as a useful adjunct to the vascular system. The author refers to its development at a later period than the external phloem as an argument in favor of its vestigial character. He regards the bicollateral bundle of the Cucurbitaceae as a compound structure consisting of two distinct vascular bundles, of which the inner has lost its xylem. He concludes that the cucurbitaceous stem represents "the vestige of a former ancestral scattered system of bundles, such as obtains in monocotyledons, of which only two series or rings remain in perfect condition, the rest appearing in the form of rudimentary external phloem strands, internal phloem strands, and medullary bundles or phloem strands."

Recently the writer learned of the appearance of a work entitled "Die Cucurbitaceen" by ZIMMERMANN (26). A copy of the work has just been received, but it is impossible at this time to make any detailed reference to any part of it. From a hurried examination, however, it is concluded that the plants therein described are entirely different from those here described, and that the investigations follow different lines. The only possible exception to this statement seems to be that ZIMMERMANN has noted the occurrence of extrafascicular bundles in *Momordica*.

Histology of axis in seedling types

The writer has studied in special detail the early histology of seedlings in *Luffa aegyptiaca*, *Cucumis sativus*, *Cucurbita Pepo*, and *Momordica balsamina*. Serial sections of each of these were made from the upper part of the primary root, through the "heel" region where the root merges into the hypocotyl, and thence upward

to varying heights along the hypocotyl. The order of structural advance and complexity seems to be in the order above indicated, and hence *Luffa* will be described first.

It is worthy of note that all of these seedlings show a pronounced tetrarch bundle system in the root (fig. 1), the usual condition observed in dicotyledons. Certain strong similarities seem to justify the grouping together of *Luffa* and *Cucumis*, and *Cucurbita* and *Momordica* for descriptive purposes.

LUFFA AEGYPTIACA AND CUCUMIS SATIVUS

The radicle of seedlings about ten days old showed a tetrarch bundle system, with two of the xylem masses slightly larger than the alternating two. This is noteworthy in relation to future procedure. In the two larger areas, three or four large, strongly marked, pitted vessels stand out conspicuously. These are surrounded by maturing wood fibers, which in turn are continuous with somewhat attenuate patches of spiral tracheae that tip the arms. The slightly smaller alternating xylem radii show a few small, somewhat irregularly disposed vessels among a few xylem fibers, while the arm is terminated by small patches of spiral tracheae as in the larger two.

About 1 mm. higher, in the center of the root, a core of delicate cells appears as a developing pith accompanied by a slight moving apart of the two pitted vessels and of their surrounding elements in each of the two larger xylem patches (fig. 2). Slightly higher, the four to six conspicuous pitted vessels with their surrounding xylem elements, seen in the two larger xylem masses, move still farther apart, while the pith tissue is considerably enlarged. As this goes on, the two smaller alternating patches become reduced to little more than two patches of spiral tracheae (fig. 3). Still higher, increase in the amount of pith tissue, continued moving apart almost to the separation of the conspicuous pitted vessels and the elements surrounding them, and a commencing division into halves of each of the smaller masses are all observed to be proceeding simultaneously (fig. 4). The four divergent pitted vessels with the xylem tissue surrounding them is shown below to represent the four divergent primary bundles in the lower part of the hypocotyl, while the spiral tracheae of the two arms and the divided spiral

tracheae of the smaller bundle masses will in time unite in front of the pitted vessels, as the normal protoxylem constituents of the stem bundles. This is shown by sections at the extreme base of the hypocotyl. Here, as shown in figs. 5 and 6, the two large xylem masses have now separated along with their surrounding elements as four distinct masses, although the spiral tracheae at the tips of the arms of these are still intact. The now split spiral tracheae, which made up the smaller alternating bundles, move toward and eventually fuse side by side with or internal to the pitted vessels and related tissue. Thus there are left two lines of radiating cells connecting the pith with the cortex. From this stage onward they become the two earlier formed of the ultimately four broad medullary rays of the lower hypocotyl. Slightly higher in this hypocotyl, the two patches of spiral tracheae in the now initiated meristeles become separated, half of the spirals in each arm moving alongside and then gradually in front of the pitted vessels, where they ultimately combine with the corresponding patch from the now broken smaller masses to constitute the internally placed protoxylem of each of the four bundles.

By this time the pith tissue has increased in amount, and the four meristeles have been pushed apart. Up to this point, the four now fully constituted meristeles show no indication of internal phloem, but have a well marked line of cambium, as well as adjacent cambiod cells, lying between the outer part of the xylem and the inner part of the phloem. From this time onward the cambium adds to the depth of xylem and phloem. About the level of the outprojecting heel, a line of nucleated cambial cells becomes continued inward around the sides and to the front of each bundle, so as to envelop each bundle in what might strictly be termed a "perixylary" cambial ring. The cells of this cambium along the sides of the bundles remain inactive for some time, but, as is indicated later, they become of great importance and functional activity in the aging stems. On the other hand, the cambium established in front of each of the four bundles starts active division, with the resulting formation of rudiments of internal phloem. Sections cut in a region slightly higher than the heel, therefore, show a well marked internal phloem to each bundle.

In the formation of this perixylary cambium, even at this early stage, phloem tissue becomes extensively developed along the lateral faces of the xylem, so that at times the bundles approach the concentric type (figs. 7-9).

At a distance of about 5 mm. above the level of the heel, a cross-section of the hypocotyl shows four widely separated bundles, each with a well developed cambium and external phloem, both of

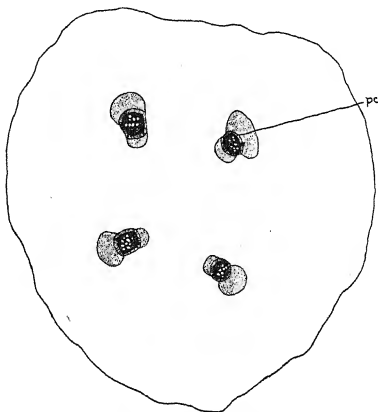


FIG. 1.—Diagrammatic transverse section of lower seedling hypocotyl of *Luffa aegyptiaca*, 5 mm. above level of "heel," showing four bicollateral meristemes each with well developed perixylary cambium (*pc*) and internal phloem; $\times 300$.

which are more or less continued inward along the sides of the xylem, and also a pronounced area of internal phloem that is being increased by the activity of the inner portion of the perixylary cambium (text fig. 1).

As cross-sections are made at higher and higher levels of the greatly elongated hypocotyl in a seedling that is six to eight days older, one of these four bundles is observed gradually to split radially, at times equally, sometimes into a decidedly smaller and

a larger half. Still higher, and usually toward the opposite side from the bundle which had just split, another bundle undergoes a similar fission. Six bundles of rather unequal size, therefore, may be observed about the middle of the hypocotyl. Still higher, one bundle after another undergoes radial fission, until ten have been established about 3–8 mm. below the insertion of the cotyledons (text fig 2). As yet it is impossible to distinguish five larger and more internally projecting bundles from five smaller ones more externally placed.

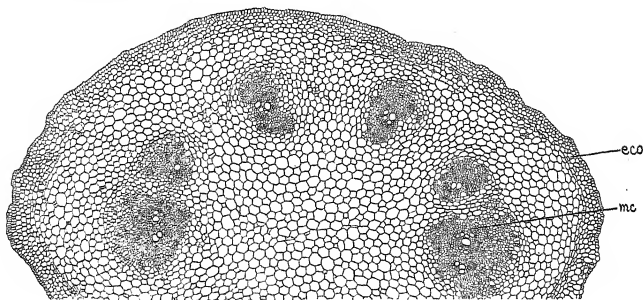


FIG. 2.—Transverse section of upper middle hypocotyl of seedling *Luffa aegyptiaca*, showing seven of ten vascular bundles: *eco*, exocortex; *mc*, medullary cambium (note radial splitting of bundles); $\times 60$.

After giving off branches into the cotyledons, the ten bundles are continued into the lower part of the developing epicotyl, but, as this lengthens and matures, five alternating bundles become decidedly enlarged and project markedly into the pith, while the remaining five show less vigorous growth and soon are of smaller size as well as more external in position.

In the development of the seedling of *Cucumis sativus*, essentially the same mode of procedure is observed as in the preceding. The monostelic mass of each radicle, as it approaches the hypocotyl, splits into two larger meristelic masses, that include all of the large pitted vessels, and two alternating, decidedly smaller ones in which

the pitted vessels are relatively smaller. Separation of the larger ones into halves and later of the smaller ones follows, and the large permanent pitted vessels are contributed by the larger meristeles, while the smaller meristeles, in their halves, contribute along one side of each meristele the small pitted vessels and spiral tracheae which, from a lateral position, gradually move toward the front of the meristeles, as do the spiral tracheae of the opposite side that were contributed by the larger meristele masses.

CUCURBITA PEPO AND MOMORDICA BALSAMINA

In contrast with the last two, the types now to be described behave in what seems to be a more balanced manner. Sections of the radicle a few millimeters below the heel show an evenly balanced tetrarch monostele. Especially to be noted in *Momordica* is a strongly developed area of hard bast external to each phloem mass (figs. 10, 11). In passing toward the hypocotyl, the four radial and similar monostelic masses gradually separate with the increasing development of pith. Simultaneously the spiral tracheae divide into pairs, which later separate, and leave between them lines of cells which connect with the pith, and henceforth constitute the primary medullary rays.

Each half-mass of spiral tracheae gradually places itself alongside of and ultimately grows in front of the pitted vessels. Slightly higher, the two patches of spiral tracheae derived from adjacent arms, converging and moving in front of the pitted vessels, unite to form a single protoxylem mass. In this case the four primary parts of the root monostele, as they rise upward to become separate bundles in the hypocotyl, retain the pitted vessels intact in position as four conspicuous masses. Splitting of the four arms gives rise to four pairs of adjacent patches of spiral tracheae. The halves of each pair become more and more separated through prolongation, from pith to phloem, of cell rows that become the primary medullary rays.

Up to this point, and even 1-2 mm. higher, each of these four bundles is solely of the collateral type, and is entirely devoid of any trace of internal phloem. About the level of the "heel," two simultaneous changes are noticed to take place in *Cucurbita Pepo*.

The bundles start in somewhat uneven manner to subdivide, so as to give rise to six, seven, and eventually eight meristeles that become fully constituted as such at, or slightly below the level of, the upper surface of the "heel." At the same time a perixylary continuation of cells from the normal bundle cambium inward to the front of each bundle develops, and quickly initiates the first rudiments of an internal phloem. All of these tissues participate in the division of the four bundles into eight. About 5 mm. above the "heel," one of the eight bundles further undergoes splitting, so that nine bundles, arranged in a ring but of somewhat varying and unequal size, may be encountered. Slightly higher, splitting of another bundle gives rise to the mature ten, seen through three-fourths or more of the length of the hypocotyl and persisting upward beyond the cotyledons.

In *Momordica* a conspicuous patch of hard bast, that almost or quite equals the soft bast in extent, is present throughout the entire length of the hypocotyl, and is continued upward into the developing epicotyl (fig. 12).

Histology of mature plants

1. *TRICHOSANTHES COLUBRINA*

Near its apex the young stem shows an epidermal layer with long glandular capitate hairs of varying length. These persist over the stem through several internodes, but are gradually shed. Internal to the epidermis the cortex shows a collenchymatous layer, rather narrow between the five strong ridges that traverse the stem lengthwise. This collenchyma swells out into five strong prominent strands that evidently form the mechanical support for the young shoots. Subjacent to this is a narrow chlorenchymatous zone, one to three cells deep, continuous around the stem and forming a mediocortex. Internal to this is an equally continuous zone of tissue that very early becomes lignified, and is two or three cells deep, the whole acting as a fibrous strengthening zone. This has generally been termed the mediocortex, but its continuity, its subsequent behavior, and the relation and aspect of the cells within that surround the bundle, all indicate that this should be regarded as a sclerenchymatous endocortex. Within it is a nearly uniform

mass of ground tissue in which are imbedded typically ten vascular bundles as island masses. Hitherto the outer part of this, which consists of about three layers, has been viewed as an endocortex. Subsequent behavior and relation to the fibrous zone without lead the writer to regard this as conjoint endodermis and pericambium, the latter in this case being of several layers, as not infrequently happens in dicotyledons.

The fundamental tissue in fresh preparations has a strikingly similar appearance throughout in its cells, whether being in position or relation an endodermis and pericambium outside, or as medullary rays between the bundles, or as pith tissue internal to these.

The vascular system consists typically of ten bundles, five smaller external ones in line with the stem ridges, and five more internal, and (in part at least) larger bundles alternating with the smaller (text fig. 3). Two of the five inner bundles are often smaller, at times greatly smaller, and of more simple structure than the other three. This peculiarity, with attendant later developments, persists into the oldest part of the stem. Each bundle shows a rather small external protophloem, a cambial and cambioid zone within, a protoxylem of one to three rows of spiral tracheae and companion cells, and most internally an internal phloem that equals or even exceeds the external in width.

In an older stage, about five internodes removed from the stem apex, several interesting and suggestive changes have arisen. By growth expansion of the stem, the epidermis and collenchymatous cortex become stretched and compressed, many of the glandular hairs tend to wither and fall, and the chlorenchyma cells of the mediocortex increase somewhat in size and deepen in color. The scleroid endocortex shows a marked change and relation, however, for it becomes ruptured, mainly in line with the outer ends of the medullary rays, largely due to proliferation of cells of the pericambium at these points. This proliferated tissue forms somewhat soft, quadrangular cells that more or less interrupt the continuity of the rounded pericambial cells. Rupture of this sclerenchymatous endocortex has doubtless also been hastened by a great increase in size of the inclosed tissue, and especially of the vascular bundles. Each bundle has developed from its normal cambium a considerable

internal patch of metaxylem composed of wood fibers, large pitted vessels, and a few simple, slightly thick walled wood cells. This cambium, on its outer face, has developed a conspicuous amount of metaphloem with prominent sieve tubes that are in connection with phloem cells. The internal phloem has also developed markedly through activity of the internal part of the perixylary

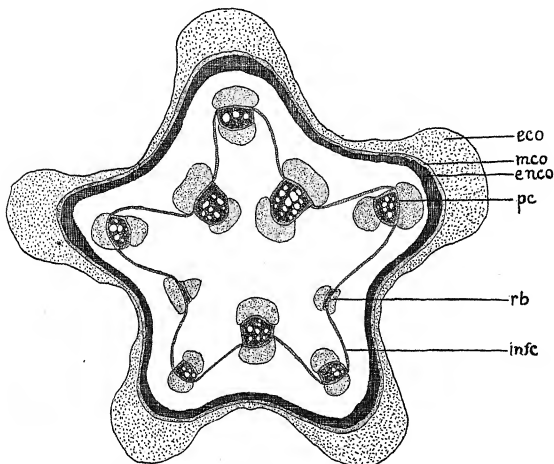


FIG. 3.—Transverse section of stem of young *Trichosanthes colubrina* near apex, showing two rudimentary inner bundles (*rb*), sinuous cambium, perixylary cambium (*pc*), and differentiated ectocortex, mediocortex, and endocortex regions (*eco*, *mco*, *enco*); $\times 45$.

cambium, or, as the writer would term it, the "prefascicular" cambium. This internal phloem in amount may be half to as much in depth as the external phloem. By this time also the protoplasmic and proteinaceous constituents of the sieve tubes have become abundant and rich in substance, so that these stain deeply with the methyl blue stain, especially where these substances are

heaped above a sieve plate. With this method of staining the continuation inward along the sides of the bundles from the outer cambium to the prefascicular cambium can clearly be traced. The lateral cambial lines are dormant in their activity as yet.

In a still older section that is 5 mm. wide, about two weeks matured from the growing apex, further advances are observed. The endocortical sclerenchyma has become still further split into more widely separated parts, owing to growth and possible proliferation of pericambial cells. Marked widening in all of the bundle zones is observed. Each as yet immature pitted vessel shows at present and retains for a long period after this a prominent nucleus and nucleolus. By this time marked inequality in two of the five larger inner bundles may be very striking, for, while the other three now show large internal phloem areas with abundant sieve tubes, a fourth has a very small patch of internal phloem, and a fifth scarcely shows a trace, or at most a very small area of six to eight cells (fig. 13). The larger three, also, have pressed strongly into the pith region. Along the sides of all the larger bundles, the lateral parts of the perixylary cambium are laying down somewhat irregular, more or less isolated, phloem tissue, so by this time it may be said that cambial activity and phloem production have already started in a somewhat discontinuous but concentric manner around each bundle.

When a slightly older stage has been reached, the three inner bundles, occasionally even the outer bundles, show progressive development of secondary medullary rays from the normal cambium. Along one or both sides of such rays a cambial line of cells has been left which may already have developed faint beginnings of lateral phloem patches (fig. 13^{mph}).

Further, the lateral phloem areas along the sides of two adjacent bundles may now be observed to have developed oblique or almost transverse interfascicular phloem bridges, linking up the lateral phloems of distinct bundle masses. Equally marked connections are here and there established between the external phloems of adjacent bundles.

By this time the upper part of the hypocotyl shows a closely similar development to the condition last described. Most note-

worthy in this region are the numerous connecting strands of phloem that constantly pass from the phloem of adjacent bundles across the medullary rays, and equally that pass from the internal phloem along the medullary rays outwardly or crosswise to join with the internal phloem of another bundle. Equally striking are the extensive transverse or oblique bridge connections established between the metaxylems of different bundles (fig. 14). Morphological and physiological connection is thus suggested for the metaxylem as well as for the lateral phloem areas of adjacent bundles.

Even more remarkable is the extensive cross connection established between the external phloems of adjacent bundles. Still another feature has now become especially prominent, although traceable in younger or higher stem sections. During development and differentiation of the cells derived from the pericambium, certain of these cells in line with each other and with the exterior ends of the medullary rays make direct connection with the most external sieve tubes of the phloem of each bundle, and also connect with and affect the cells of the soft mediocortex, so that these become modified into that system of sieve tubes which FISCHER (3) has designated "extra-cyclic sieve tubes." By this time the stem has matured through three weeks to a month of growth. About two feet removed from the apex, such growth has proceeded in the phloem of the axis that not only is there an extensive internal and external phloem, but lateral phloem masses have formed along the sides of the metaxylem which may almost or quite connect the external and internal phloems. These lateral areas by oblique or transverse diverticula, crossing the medullary rays, may directly connect with one another. Like connection between the phloem of adjacent bundles is even more extensive, while external ramifications from the external phloem into the mediocortex are fully established.

It will be seen from fig. 13 that the condition of bundle inequality already noted in the young stem has persisted and become greatly accentuated in the nearly mature plant, that is, in the older part of its stem.

POTTER (11) calls attention to the formation of secondary medullary rays in the plants he studied and regards their presence

as a somewhat noteworthy feature in the history of cucurbits. In *Trichosanthes* and other short-lived herbaceous types, however, secondary medullary rays are formed by the bundle cambium in one to three of the stronger bundles at least. Not only so, but in the largest bundles of a four to five months old stem, tertiary medullary rays of fair depth may have developed. The later formed medullary rays pass out to phloem tissue of similar age. In this manner, all parts of the increasingly deepening xylem of the bundles are kept in close contact physiologically with the elaborated sap passed down through the external phloem.

As transition is made to the oldest and thickest part of the stem in a mature plant, the structure is fundamentally the same as that already detailed. As one approaches the region of the decayed cotyledons, the interfascicular xylem and phloem connections have become still more extensive and intimate. The secondary changes exhibited in the hypocotyl, as compared with its structure in the seedling state, are the most conspicuous features in the history of the plant, and are of such a nature as to cause one, somewhat metaphorically, to liken it to the central exchange in an extensive telephone system.

A hypocotyl that is from two to three months old shows considerable reduction in size of its sclerenchymatous endocortex. This appears now only as small and scattered patches of lignified tissue. The bundle relations are of great and special interest, for, as compared even with the lower part of the epicotyl, this region shows a widespread ramification of sieve cells that extend from the external phloem outward into the mediocortex; at times, even to its outer margin. Through pericambial activity, mainly in line with the outer ends of the deepening medullary rays, abundant sieve cell connection is established between the external phloems, so that not infrequently these may be linked together into one common phloem system. In addition, during the steady growth of the hypocotyl, abundant bridges of sieve tubes pass inward from the normal phloem; in part to interconnect from bundle to bundle, and in part to establish connection with masses of lateral phloem already developed along the radial faces of the bundles. These lateral patches form frequent interconnections across the medullary

rays, and not infrequently show connections with the internal phloem. In passing, it may be observed that one or more of the bundles have developed such cambial activity along the one or both sides of the xylem, that a broad plate of typical phloem may arise that extends continuously from the internal to the external phloem.

As passage is made toward the lower part of the mature hypocotyl, the elements of the wood exhibit fewer lignified elements, so that in some bundles patches of wood fibers surrounding one to four pitted vessels may be imbedded in the midst of an extensive area of thin walled parenchyma. Furthermore, the entire sieve tube system now reaches a climax of complexity, abundance of sieve elements and branchings. Correlated with this are the frequent cross connections between the xylems of adjacent bundles, and in these the pitted vessels, exposed in their pitted faces, form striking objects.

As transition is made to the summit of the mature root, the interlacing connections become still more complex, so that a confused tissue pattern only is discernible. Especially is this so if one or more side rootlets start from the main root. While this is proceeding the lateral plates and internal phloem masses of the perixylary system gradually break up and retreat toward the exterior, the latter having previously come together as a central mass of tissue. Deeper down this thins out, leaving in the lower part of the root six to four bundles that meet in the center by their protoxylems, but which externally have become more and more deeply separated by the increasingly wide medullary rays.

2. LUFFA AEGYPTIACA

Study of soft young stems of *Luffa* about a foot in height, about the second epicotylary internode, shows a sharply pentagonal outline, with epidermis, collenchymatous, chlorenchymatous, and as yet immature sclerenchymatous cortical zones continuous around the stem. Imbedded in the fundamental tissue are typically ten bundles, five of which are internal and five markedly external. As in *Trichosanthes*, however, it frequently happens that at least one of the internal bundles is decidedly smaller than the other four, a

peculiarity which may persist into the mature stem. Already, far down in the hypocotyl, a prolongation inward of the perixylary cambium has on its inner face given rise to an internal phloem area for each seedling bundle, but in this type a semi-crescentic internal phloem has already originated that may be half as large again as the flat external phloem. Frequently, also, the lateral rows of cambial cells that connect the external and internal cambiums are readily discernible. Thus again is constituted a perixylary sheath. The young bundle apart from this deserves no special description.

If now, as for *Trichosanthes*, successive sections be made of a mature plant about four months old, starting with the third or fourth internode from the apex and gradually proceeding backward, the following features might be emphasized. The stem outline is not merely pentagonal as in the seedling, but, owing to luxuriant development of the collenchymatous cortex along five lines, it exhibits five ridges that alternate with five grooves. The lignified endocortex has already developed thickened walls in its five to seven layers. The one small inner bundle usually shows no internal phloem at this stage. A second bundle of the inner five may also be reduced in the amount of xylem and phloem, and not infrequently has no internal phloem. Sections made about the eighth internode from the growing apex still show a continuous zone of lignified endocortex, but in relation to the future behavior of this tissue and the outward passage of sieve tubes into the mediocortex, it should be noted that narrow radial patches of richly protoplasmic cells grow into the six inner lignified cell layers of the endocortex, thereby leaving for a time only one or two external lines in continuity.

A feature shown at this time in *Luffa* is the formation of a continuous cambium ring of highly sinuous course. As shown in fig. 15, one can readily trace between any two bundles three to four flattened, highly protoplasmic nucleated cell rows, which are continuous with similar cells within the bundles. The central row is a cambium, while those on either side are young cambioid cells. This histological detail bears out and further extends POTTER's findings in *Cephalandra* (11), that the five outer and five inner bundles are the slightly displaced members of one ring, typical for dicotyledons. This also negatives WORSDELL's (25)

hypothesis that primitive cucurbits started with numerous irregularly disposed bundles.

Sections made through the twelfth to fifteenth internode from the apex show a greatly stretched epidermis, exocortex, and medio-cortex as a narrow zone. The endocortex has become broken up into lignified patches immediately exterior to each bundle. Marked growth has taken place in all of the vascular bundles. The striking difference of the interfascicular cambial cells as compared with those of the intrafascicular stretches deserves special notice. While the latter are small, flattened, and truly cambioid, the former are large, finely protoplasmic, and nucleated cells of quadrangular outline but with extremely thin walls. By subdivision of these, cells arise externally and internally which add to the depth of the medullary rays, and also to the lateral lines of perixylary cambium. The lateral portions of the perixylary cambium, as in *Trichosanthes*, have now started considerable lateral additions to the phloem, especially along the sides of the larger and deeper bundles (fig. 16). Secondary medullary rays, about ten to twelve cells in width, have been laid down, and these divide more or less completely the original phloem of each bundle into halves. The internal phloem masses have so grown and pushed inward that the area formerly occupied by the pith is now filled. Occasionally, also, oblique sieve tubes and companion cells may stretch outward from one or more of these to connect with the corresponding elements of the medullary phloem.

Between the last condition of stem section and the mature epicotyl one or two internodes above the level of the cotyledons, important growth activities have taken place. Each bundle, with the possible exception of the original inner one of the five, is now a broad wedge-shaped mass, bounded on either side by the primary medullary rays and the lateral zones of perixylary cambium. Deep secondary medullary rays, tertiary, and occasionally short quaternary medullary rays extend into the phloem. All of these are about eight cells in width. The outstanding features of growth, however, are now centered in products of the lateral and prefascicular cambial tissue. As compared with the rather limited amount of medullary phloem described in the previous paragraph, the lateral cambium

has actively proliferated additional phloem tissue, generally in localized masses, and also has cut off cells that simulate, and evidently function as medullary plates at right angles to each primary medullary ray, termed by the writer "admedullary rays." Not only so, but secondary lateral cambiums, peculiar to each patch of these, have laid down internally masses of tertiary xylem of varying size, composed in great part of thick walled fibers, but including also one to four pitted vessels. These tertiary bundles produced by the perixylary cambium the writer has designated "transmedullary bundles." Even more remarkable are the changes effected in connection with the internal phloem masses. Each of the nine or ten prefascicular cambiums, that formed for a time only internal phloem, has laid down between itself and the protoxylem a deep mass of tertiary xylem. Thus are formed the obcollateral bundles (text fig. 4). Furthermore, the xylems of the transmedullary bundles and the xylems of the internal obcollateral bundle masses throw out frequent oblique or transverse branch connections that fuse with one another.

Toward the base of the secondary medullary rays, a limited amount of cambial activity gives rise to small areas of sieve tubes and companion cells, termed "intraxylary phloem" by the writer. The result of this enormous internal growth activity is that the original pith tissue or pith cavity becomes completely filled up with large internal obcollateral pith bundles, which, however, still retain connection with the protoxylems of the bundles.

Although up to this point no attention has been called to the external ramifications of the sieve tubes and companion cells in *Luffa*, it should be said that even in sections of the young stem like the second or third described, ramifications are sent out from the external phloem which at times cross to make connections with adjoining bundles on either side, but also, passing outward, begin to unite with cells of the mediocortex or to insinuate themselves between the masses of broken up and now discontinuous endocortex. Resemblance, therefore, to the conditions already described in *Trichosanthes* is perfect.

In passage downward through the hypocotyl (which may be nearly an inch in diameter) conditions become even more exagger-

ated and complicated. More and more copious anastomoses of the external phloem patches may be effected with each other, and may abundantly be sent out into the mediocortex. Longitudinal, oblique, and radial diverticula may also connect the xylems of the

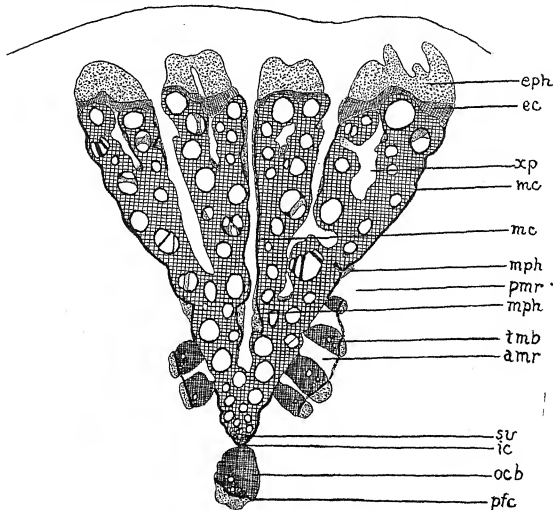


FIG. 4.—Transverse section of hypocotyl of mature *Luffa aegyptiaca*, showing vascular bundle whose internal phloem has become obcollateral bundle (*ocb*) by addition of secondary xylem produced by prefascicular cambium (*ic*); inner areas of medullary phloem by addition of secondary xylem produced by sections of medullary cambium have become transmedullary bundles (*tmb*), separated by admedullary rays (*amr*); medullary phloem formed along secondary and tertiary medullary rays (*mph*) by medullary cambium (*mc*); $\times 16$.

ten bundles. The transmedullary bundles here reach their greatest size, as do the admedullary rays between them. The obcollateral bundles that primarily originated as internal phloem masses now frequently become wholly separated from the protoxylem.

Finally, as transition is made to the root, thinning out and disappearance of these bundles takes place, and equally of the transmedullary bundles, until, in the root proper, four convergent wood wedges unite with each other by their internal faces or apices.

Thus it is evident that the hypocotyl is the most complex region of the axis, and in the extensive and intricate ramifications and unions of the separate bundles (equally in their phloem and xylem constituents) there is a remarkable provision made for the passage of abundant crude sap upward along the xylem, and of elaborated sap downward in the greatly developed phloem.

3. CITRULLUS VULGARIS

This excels all other members of the family in length of axis, in luxuriant growth of side branches, and in size of hypocotyl and immediately connected region above and below. Sections of the main axis or of a side branch, about three internodes removed from the stem apex, in a plant about four months old, show fundamentally similar detail to that of *Luffa* in its epidermis and cortex, except that the continuous lignified endocortex is here only two or three layers deep. Internal to the endocortex is a thin walled epidermis of rounded cells, and internal to this in turn is the pericambium.

The same inequality in bundle size that has previously been described for *Luffa* is observed in *Citrullus*, and each bundle develops the typical perixylary cambium. Ten radiating groups of thin walled cells at the back of and in line with the ten medullary rays are also richly protoplasmic, and mark the future areas where abundant cambial growth in and around them will cause splitting of the lignified endocortex. The pith tissue in *Citrullus* early breaks down in large measure.

While account has not been taken in any of the preceding descriptions of the presence of tyloses, it might now be said that this is an extremely common and striking feature in most of the cucurbits studied. The large pitted vessels in the earlier stages of their maturation frequently become invaded by surrounding thin walled cells. Each tylosis cell is provided with active protoplasm, and a large evident nucleus, and a shining nucleolus. These cells evidently

remain for a considerable period as a living, spongy pseudoparenchyma that more or less fills up the vascular cavities. Of all the types studied in this family, *Citrullus* shows this feature most strikingly. Already, in the young sections just described, the inner two or three of the large pitted vessels among the eight or more now formed show extensive tylosis. Gradually, as the bundles increase in depth and additional pitted vessels arise, similar tylosis is seen proceeding in them. It seems to the writer, however, as if certain of the vessels in the younger stems were exclusively or mainly invaded in this manner, but with increasing age a progressive development of tylosis goes on, so that in stems about four months old and about an inch in diameter, nearly all of the now abundant pitted vessels are blocked up with tylosis tissue. Moreover, if one may judge from the staining reaction of the protoplasmic contents and the persistence of the nuclei, this tissue seems to remain alive and may importantly function in the distribution of crude, and may be even of elaborated sap.

In sections taken through the eighth to tenth internode, the radiating groups of cells, described as projecting into the lignified endocortex, have so enlarged and multiplied that they have caused the endocortex to break up into ten distinct sclerenchyma plates. A large celled interfascicular cambium is now fully established, and from this time on steadily cuts off large medullary ray cells on its inner and outer faces. Of the large inner bundles, a fourth in addition to the preceding three may be nearly or quite equally developed, while an extreme rudiment of the fifth (that appears only as a small patch of phloem tissue) may still remain.

The lateral portions of the perixylary cambium may already have formed a slight amount of lateral medullary phloem. As a rule this consists only of undifferentiated cells or patches of smaller, somewhat angular phloem cells. Occasionally one can observe one or more sieve tubes, which represent the beginnings of what later become the transmedullary bundles.

Selecting next an internode that is a centimeter in diameter, one observes immediately external to the bundle phloem two layers, occasionally widening to three, of rounded cells that represent endodermis and pericambium. Between the lateral exteriors of

the bundles and the extremities of the rapidly deepening medullary rays, proliferation of the pericambial tissue has given rise to as many as eight layers of more or less cubical cells. The vascular bundles have increased markedly in size, and, if the stem or lateral branch has grown rather freely on all sides, the five inner bundles may all be represented as well as the five outer, for, as discussed more in detail under the physiological section of this thesis, the relative size to which the inner five bundles of the axis may attain seems to be largely conditioned by the dorsiventral relations or by mechanical strains along the plant axis. As a rule, four at most of the five inner bundles are almost or quite equal in size, a fifth being often decidedly smaller and more external. The inner bundles also are on the average three-fourths to as much again larger than the outer bundles. The external phloem is now greatly increased in amount over the internal, being three to five times greater.

The sieve tubes in *Citrullus* are probably the largest and finest studied by the writer. Those of the external phloem are on an average as large again as those of the internal phloem. The external and internal segments of the perixylary cambium are more strikingly regular and cambium-like than in any other type studied. From the latter portion, a steady amount of phloem is laid down by differentiation of the cambioid cells. Furthermore, the commencing formation of lateral transmedullary bundle rudiments as described in the previous paragraph has considerably advanced. It is noteworthy, however, that at the present stage, and even in somewhat larger and older axes, transmedullary bundle development only proceeds along one side of two or three of the inner bundles (text fig. 5).

The earliest formed and internal part of the xylem of *Citrullus* consists almost wholly of indurated wood fibers and large pitted vessels that soon become invaded by tylosis cells. As compared with other types already studied by the writer, however, from the age of about a month onward the xylem becomes soft and largely cellular, with only scattered irregular masses of indurated elements and pitted vessels. This cellular condition is due in part to the rather irregular and broad spreading character of the secondary and succeeding medullary rays, but in largest measure it is due to

extensive areas of xylem tissue that remain in the parenchymatous state instead of becoming fibers or large vessels.

Up to this stage little, if any, indication has been shown of connections between the external phloems or other tissue of the bundle. Some of the cambioid derivative at the ends of the medul-

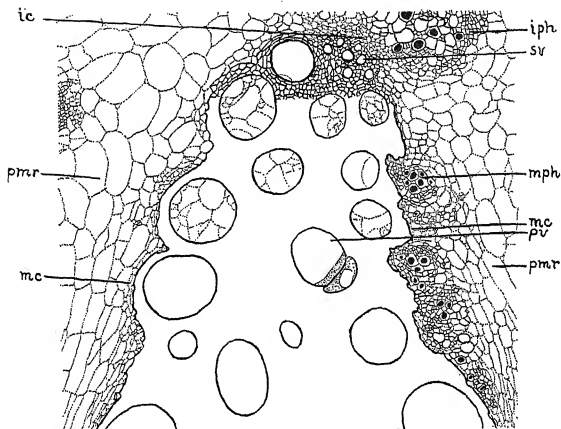


FIG. 5.—Transverse section of branch of mature *Citrullus vulgaris* near hypocotyl; inner part of one bundle showing large areas of medullary phloem (*mph*) formed by medullary cambium (*mc*) along lateral faces of metaxylem of bundle; note almost entire absence of medullary phloem on opposite face of xylem: *ic*, prefascicular cambium; *iph*, internal phloem; *sv*, spiral vessels; *pmr*, primary medullary ray; *pv*, pitted vessel (details of xylem tissue omitted except at extreme inner part); $\times 50$.

lary rays shows conversion into sieve cells with well developed sieve plates, that often in one place appear on edge, in another oblique, or again in full face expression with the sieve pores perfectly exposed. From this stage onward a steadily progressive advance in such oblique or transverse anastomoses may be traced; but further, the beginnings of differentiation in cells that connect

with the mediocortex constitute the first beginnings of FISCHER's ectocyclic phloem (3).

Passing next to stems or branches that are about 1.5 cm. in diameter, the deep development of secondary, tertiary, and even at times of beginning quaternary medullary rays tends to cause separation of the external and hitherto continuous phloem areas into two, four, or even six separate patches. Owing, however, to what the writer at present only can regard as a growing capacity in sieve tubes of the bundles and in medullary ray cells between the phloem masses, more and more extensive connections pass from phloem mass to phloem mass, and also outwardly into the cortex, so that FISCHER's ectocyclic phloem is now in active process of differentiation. In the meantime, the internal phloem areas have been rapidly increasing in size through activity of the prefascicular cambium, but greatly more striking is the rapid increase now proceeding in the medullary phloem of nearly all the bundles. Along the sides of the secondary and tertiary medullary rays, or in cellular pockets left undifferentiated in the metaxylem, isolated phloem groups have appeared in quite irregular and scattered manner (fig. 17).

The highly parenchymatous nature of the xylem, as well as the irregular outlines of the secondary and later medullary rays, has now become a pronounced feature of the stem. It might be added that a steady formation from within outward of vascular tylosis tissue has so proceeded that a large number of the pitted vessels are now extensively filled. The extensive development that has gone on in the internal phloem areas, particularly in those of the inner bundles, has now caused extensive filling up of the area once occupied by the pith cells.

In still older stems, about the third to the fifth internode above the cotyledonary junction point, the stem diameter may be 2-2.5 cm. Extensive transverse bridges between all of the external phloem masses have now been effected through continuous growth of the phloem cells and sieve tubes from one bundle toward another. Into the numerous zones of cells that lie internal to the lignified endocortex, abundant ramification and prolongation have been effected, so that it can truly be said that there is now a broad

continuous zone of external phloem, made up of vertical, oblique, and transversely connected sieve tubes interspersed with companion phloem cells.

The only exception to this statement is where the large cells across the outer part of the primary medullary rays have so persisted in their function of distributing the elaborated sap transversely that few, if any, of the connecting phloem strands have succeeded in differentiating amid this transversely conducting cellular expanse. It might also be noted that the total bulk of phloem equals one-third to one-fifth that of xylem, account being taken of the zone of external phloem, the important lateral medullary phloem, and the eight to ten internal phloem masses. This is in marked contrast with the relative amount of phloem and xylem even in ordinary herbaceous plants, not to say in shrubs or trees, so that it seems to be another correlated and necessary factor in the enormously rapid growth and vegetative luxuriance of this plant. This question is discussed from the physiological standpoint later.

Such a condition is entirely in keeping with the extremely rapid growth and high nutritive capacity shown by *Citrullus*. About this level, also, bridges of xylem are occasionally laid down, connecting adjacent bundles, although as yet these are not nearly so numerous or wide as in the hypocotylary region next to be described. The accessory intraxylary phloem patches now have become more abundant and larger in extent.

The hypocotyl in a mature plant (one about four months old) measures about 3.5 cm. in diameter, and 15–20 cm. in length. The external phloem has now reached its maximum of growth, and through formation of successively intercalated secondary, tertiary, and succeeding subsidiary medullary rays, the original primary phloem mass has split up into 16–24 phloem wedges of varying size. These daughter phloem areas all communicate with one another by oblique or transverse sieve tubes, as well as by companion phloem cells. In the peripheral portion of the hypocotyl especially, the copious anastomoses of sieve tubes are most striking, and frequent passage of them between the peripheral sclerenchyma patches can be seen to take place around the entire axis. It is in the region occupied by the xylem tissue or the area

internal to it, however, that the climax changes have been effected that are now to be recorded.

The xylem in the hypocotyl region shows a more extensive cellular structure than does the oldest or any other part of the epicotyl. Frequent patches of two to six large pitted vessels surrounded by thickened wood fiber elements may be wholly surrounded by thin walled parenchyma. The formation of tylosis tissue within the vessels has here reached a climax. It is in the marvelous development of perixylary and intraxylary phloem, however, that the hypocotyl is preeminently distinguished. As to the former, one may notice that just beyond the huge patches of internal phloem there have developed frequently from twelve to twenty lateral medullary phloem masses, through the activity of the lateral sections of the perixylary cambium. In many cases, especially toward the interior, these phloem islands may be quite separated from one another by admedullary ray cells running at right angles to and meeting with the primary medullary rays. More externally, however, instead of isolated phloem areas, deep tracts of phloem may be intercalated between a primary medullary ray and a xylem face.

The feature that eminently distinguished this plant from all other types studied consisted in the luxuriant development of intraxylary phloem in a most irregular and scattered manner through the xylem parenchyma or along the lines of the tertiary and subsequent medullary rays (fig. 17). The total amount of such tissue in each of the xylem areas varies considerably, and seems determined in part by the position of the bundles in relation to the strongest radiating branches in the epicotyl above. One wood-wedge may have rather small and scattered patches toward one side of the area and extensive masses toward the other. Some wedges may be rather wanting in this tissue, or have only small patches of it; while an adjacent xylem mass may have large and abundant phloem groups. Furthermore, the sieve elements vary considerably in size; some small sieve cells with transverse plates show only about ten pores, others again may almost equal the sieve plates of the outer phloem, and then show fifteen to thirty pores. Collectively the amount of accessory phloem thus developed must form a

valuable auxiliary to the external and internal areas. Here it may be added that these phloem patches not infrequently form anastomoses with each other.

Turning now to the internal phloem, each of the four or five masses belonging to the inner bundles has increased in the hypocotyl to an enormous degree, and even those of the four or five outer bundles are of large size. The inner ones deserve special attention, for in addition to retaining in an active state the prefascicular cambium that normally functions in the epicotyl, and which here has given rise to huge internal phloem tracts, a crescentic line of cambial tissue has developed around the inner face of each internal phloem area, which lays down a crescentic mass of xylem either as a continuous mass or as two or more masses that are separated by short medullary rays.

In transition from the thick hypocotyl to the root, it usually happens that several rather strong secondary roots pass off from the primary so that the size of the latter is quickly reduced. In nearly every point of structure, however, the details revealed in the hypocotyl are continued into the root tissue. Not the least conspicuous in this connection may be mentioned the wide and luxuriant peripheral phloem zone, the highly cellular xylem, the abundant vascular tyloses, and the rich intraxylary phloem. The highly complex internal phloem patches previously described gradually thin out and are absorbed, and contemporaneous with this the xylem wedges come together to form a continuous central tissue.

In brief summary of the details shown by *Citrullus vulgaris*, it might be said that it greatly excels all other types studied in the large size and soft succulent character of the xylem masses; in the wide, irregular, and abundant medullary rays (secondary, tertiary, etc., included); in the extreme size of the normal phloem; in the abundant anastomoses that prolongations of these bundles form between one another and into the endocyclic cellular tissue as well as (although to a less degree) into the ectocyclic; in the extreme activity of the entire perixylary cambium; in the huge internal phloem masses with their ultimately complicated obcollateral bundles that result from the activity of the prefascicular cambium; in the marked activity of the lateral perixylary cambial stretches

with resulting formation of transmedullary bundles; but most of all in the often extensive patches of intraxylary phloem that must add greatly to the capacity for rapid transfer and lengthwise distribution of elaborated food along the lower part of the stem axis and all parts of the root system. When one surveys the collective development and capacity of these constituent elements, it is safe to say that *Citrullus* greatly surpasses any other type of rapidly growing herbaceous plant. It is throughout the length of the hypocotyl that these complexities reach a climax of development.

4. LAGENARIA VULGARIS

Sections of the lower middle hypocotyl of *Lagenaria vulgaris* reveal differences in structure that are not shown in any of the preceding types, and which persist from the young state up to and including mature growth. Such a hypocotyl, in a plant about 18 inches in length, shows an external epidermis abundantly provided with short pointed hairs. Underneath this is a rather discontinuous zone of colloid exocortex, but, in contrast with *Citrullus*, *Luffa*, and *Trichosanthes*, a sclerenchymatous endocortex is here wholly wanting. The endodermis and pericambium together consist of two to four soft, thin walled cell layers, the inner of which lies directly against the bundle system. The bundles here are eight in number, and are disposed in a single ring, although four that alternate with the other four are recognizably larger and project slightly more into the pith. Furthermore, two of these inner bundles are decidedly larger than the other two, indicating that each of these will split radially and so give rise to the full complement of ten, seen in the upper part of the hypocotyl as well as typically in the epicotyl.

Each young bundle of the hypocotyl in the age indicated exhibits a patch of hard bast, the lignified elements of which readily absorb safranin stain and assume a bright scarlet tint. The zone of perixylary cambium can readily be traced around each bundle, and may even develop at this early stage slight indications of lateral phloem. A large lysigenous cavity exists in the pith, and that is bounded by two to four continuous zones of pith cells.

Very early in the growth of the hypocotyl and young epicotyl, crescentic cambioid cells originate that join on with the intrafascicular cambial sections. By this union a highly sinuous although continuous stem cambium can readily be traced. Through rapid division of the constituents, the medullary rays from this stage onward are rapidly deepened.

In passing toward the upper part of the hypocotyl, the two larger inner bundles undergo longitudinal splitting, and from this point upward ten bundles are typical. It not infrequently happens that one bundle becomes feeble in growth, or is even eliminated, but in connection with these large bundles, and of extreme interest in relation to the structure of the epicotyl as contrasted with the hypocotyl or the root, there appear external to them two crescentic endocortical masses of sclerenchyma. These are the beginnings of a typical endocortical sclerenchyma ring formed by gradual spreading of the two patches outward on either side, as transition is made to the epicotyl. It will thus be seen that the hypocotyl and epicotyl contrast markedly in two histological details. The eight to ten hypocotylary bundles each possess a strong heavily lignified patch of hard bast fibers, which are lacking in the bundles of the epicotyl. The hypocotyl, also, is wholly devoid of lignified endocortical ring, except for a rudiment toward the summit of the hypocotyl; while the epicotyl, throughout its entire maturing history, shows a continuous sclerenchymatous endocortical ring, which gradually splits into patches with the increase in size of the stem.

Sections through about the third or fourth internode from the growing apex show a continuous sclerenchyma ring that is about four cells deep. This ring surrounds an endodermis and pericambium of two to four layers of rounded cells. The perixylary cambial tissue that surrounds each bundle stands out prominently. Each internal phloem patch may be about the size of the external, or from such to about three-fourths as large. The lysigenous pith cavity of the hypocotyl has been continued upward, and is here as pronounced as in early growth of the seedling.

About the twelfth to the fifteenth internode from the growing apex, the endocortex has split into patches of varying size. Deep

secondary and less deep tertiary medullary rays have been inserted, and passing outward separate each external phloem area into four minor patches. Along the inner ends or sides of these minor rays a limited amount of intraxylary phloem forms, but this never compares in amount with that already described for *Citrullus*. Through activity of the lateral sections of the perixylary cambium, deep plates of medullary phloem arise that may stretch through three-fourths the depth of the adjoining radial faces of the xylem, but, as in most of the types studied, very unequal amounts of this tissue may arise on opposite sides of any bundle xylem.

In *Lagenaria*, each internal phloem patch has attained quite a large size during this stage, although in comparison with the external, the internal is only about one-sixth to one-eighth the area. Through steady expansion of the circle of pith cells and of the central lysigenous cavity, these internal phloem masses stand apart from all other constituent tissues of the region.

On reaching the upper part of the mature hypocotyl, the same climax complexity of structure is seen that characterizes those types already described. The pericambial tissue has divided to the extent of forming at least four encircling layers of cells. The isolated hard bast areas have somewhat spread out into oval masses. Each bundle patch of external phloem is now so extensive as almost to equal in size the collective mass of xylem.

Probably in no types studied does the perixylary cambium show more clearly than in this plant, and especially in its hypocotyl. Connecting externally with the normal cambium, it winds along the sinuous edges of the bundle, and in the mature hypocotyl not infrequently forms on its outer face a continuous mass of phloem that unites the internal with the external phloem. Unlike *Citrullus*, intraxylary phloem is here very feebly developed. The internal phloem areas are oval to circular in outline, and attain to rather a limited growth. So far as observed, neither accessory cambium, internal xylem, nor sinking in of the phloem patches into the pith takes place. Tylosis in the pitted vessels is of rather rare occurrence in this type.

In passing from the hypocotyl to the root, the internal phloem masses gradually attenuate in size, while the bundle apices fall together, but the extensive lateral medullary phloem plates per-

sist. Still lower, the eight bundle masses fuse into six and eventually into four wedges, while the lateral medullary phloem diminishes in amount.

This accessory tissue tends to persist for a considerable distance downward in the primary root as shallow irregular sheets of phloem bordering the faces of the xylem tissue.

5. MOMORDICA BALSAMINA AND M. CHARANTIA

Sections made through about the fourth internode of a mature stem show a structure essentially the same as that already described for the upper part of the hypocotyl and the lower part of the epicotyl of the seedling. The ten (or occasionally nine) bundles usually show five larger inner and five alternating smaller ones, that may be externally on a level with the larger bundles or may slightly project beyond them. In this genus the cortex consists at most of only four layers of cells, entirely lacking in lignification. Internal to the cortex is an endodermis surrounding a pericambial layer, both of which lie immediately against the strong patches of hard bast peculiar to the bundles. The pericambium deserves emphasis here because of its significance in the future increasing growth of the stem. In *Momordica* a line of development and of secondary increase takes place which is entirely different from anything that the writer has observed in the other genera of Cucurbitaceae. It should be noted that internal to the level of the hard bast, and on either side of the lateral angles of the soft bast, two groups of cells undergo considerable lignification. Since these arise near the exterior corners of the soft bast, they might be termed accessory hard bast. Occasionally, however, only one may develop or both may be absent. Generally they are very conspicuous and persistent structures.

Above this stage also, along the lines of the medullary rays and inward even to the pith, practically every cell begins to show the rudiments of crystals, that may vary from prismatic to conglomerate in shape. Only along the lateral lines of the perixylary cambium are the crystals extremely small or wholly absent.

Here the perixylary cambium is highly conspicuous. The activity of its lateral stretches is greatly less than in other genera studied. The reason for this is seen in the unique mode of secondary

growth. The internal phloem is usually developed. Occasionally a smaller one of the five outer bundles may be largely or wholly devoid of it. In slightly older stem sections, such as the seventh internode from the apex, a striking new formation can be observed in its incipient stage. Immediately external to two to four of the hard bast patches, the zone of pericambial cells starts division. By the time this phloem tissue is about seven layers deep, its most external layers begin to show lignified thickening, and constitute a hard bast to each of these incipient "extrafascicular" bundles, as the writer proposes to designate them.

In still older sections the zone of pericambial cells outside the hard bast of each normal bundle has indirectly added a certain amount of xylem between itself and the normal hard bast. This apparently is due to the formation of a separate layer of cambial cells, that possibly may represent the inner zone of the extrafascicular bundle phloem tissue, or a special bundle cambium separated from the pericambium.

Following the growth of these bundles into older parts of the stem, as many as ten extrafascicular bundles, one beyond or external to each bundle, may have arisen (fig. 18). As in other genera, the most exaggerated development that the writer has been able to follow lies in the hypocotyl. Here there seems to be a correlating and great distributing center alike for crude and for elaborated sap. While all of the normal bundles in this section showed an extrafascicular bundle, that figured (fig. 18) greatly excelled the other six in the hypocotyl of the plant. The bundle itself lies external to a mass of normal hard bast, that here has split into three now separated patches. Immediately external to the normal hard bast is a zone, that at present can only be called a proliferated pericambium. Beyond this is an extensive plate of xylem traversed radially by two primary extrafascicular bundle medullary rays, and by one or two similar secondary ones. This woody tissue resembles that of the normal bundles; however, as seems to be true of all secondary bundles, the xylem is here wholly devoid of spiral vessels. External to the wood is a zone of five or six thin walled cells, with deeply stained nuclei that together represent the extrafascicular cambium and cambioid layers. Beyond this is

the first formed phloem tissue of these bundles, recognizable in its two parts, an inner or soft bast with large sieve tubes and companion cells, and an outer and now somewhat split hard bast of two or three irregular layers.

In the outer part of the bundle an additional patch of phloem has arisen immediately external to the hard bast just noted. This as yet consists only of soft bast, made up mainly of phloem cells, but with some distinct indications of sieve tubes. Differentiation of two of the outer of these into an additional hard bast has been observed. Finally, an even greater advance than the last is seen in one extrafascicular bundle, where a third soft bast has originated outside the hard bast of the second phloem zone. The areas encountered from without inward to the pith tissue are as follows: (1) epidermis; (2) cortex of large soft cells, two or three layers deep; (3) third or most external soft bast of the extrafascicular bundle; (4) second or most external hard bast of the extrafascicular bundle; (5) second or median soft bast of the extrafascicular bundle; (6) first or most internal hard bast of the extrafascicular bundle; (7) first or most internal soft bast of the extrafascicular bundle; (8) cambium and cambiod tissue of the extrafascicular bundle; (9) xylem (traversed by medullary rays) of the extrafascicular bundle; (10) one to three layers of thin walled cells, endodermis-pericambium (?); (11) hard bast of the normal bundle; (12) soft bast of the normal bundle; (13) intrafascicular cambium and cambiod tissue of the normal bundle; (14) metaxylem of normal bundle; (15) protoxylem of normal bundle; (16) prefascicular cambium (internal section of perixylary cambium); (17) internal phloem; (18) pith.

The writer believes that plants of this species, if vigorously grown and fully nourished, would show an even more evolved formation of tissues than anything described in this paper. He hopes to carry out such studies in the near future.

The crystals already mentioned in their earlier formation have now reached a climax of size and abundance. They show every possible transition, from single tabular forms with straight faces to complex conglomerate types with numerous facets and points.

In the allied species, *Momordica charantia*, an exactly similar formation of extrafascicular bundles takes place, but so far as

observed, they are both fewer in number and more limited in growth capacity, for seldom are there more than two or three of these bundles formed around the stem. More detailed study would be advisable here.

The two species of *Momordica* studied in comparison with all of the other types suggests that this genus has early diverged from some more primitive group which had already developed bicollateral bundles, but had not reached the stage where some of the more complex phloem formations had started. The production, therefore, of a gradually increasing number of extrafascicular bundles that could simulate in their activities the medullary and intraxylary systems, as well as the endocyclic and ectocyclic phloems as auxiliary distributors of elaborated sap, is not without significance. In all of the material available, however, these extrafascicular bundles do not seem to pass higher than the third to seventh epicotylary internode, depending upon the age of the plants.

6. CUCURBITA PEPO

The earlier stages of growth conform very closely to *Citrullus*. The later stages, and particularly the structure and growth of the mature hypocotyl, deserve some consideration. When one thinks of the huge fruits borne by the vines of *Cucurbita*, which are matured in six to eight weeks from the time of flowering, it is not surprising that in stems about a month old the phloem often exceeds the xylem in total amount, especially in the large inner bundles, in the ratio of 3:2. While this ratio is diminished later, it still remains true that the total amount of phloem tissue equals or very nearly equals the xylem.

In the lower internodes of fairly mature stems (ten to twelve weeks old), the bundles consist of loose xylem tissue that is deeply divided by secondary, tertiary, and quaternary medullary rays, and the vessels become abundantly filled by intruding tylosis cells, but two features only need be emphasized here. (1) The perixylary cambium, as in other types, in the lower part of the hypocotyl quickly started to form medullary phloem, in part in patches separated by admedullary rays, along the more internal lateral faces of the bundles, and in lines of phloem along the more

external radial faces. The process continued until, in the hypocotyl of stems about four months old, there may be considerable masses of xylem on the inner sides of these, and so, not infrequently, one may count five or six complete collateral transmedullary bundles toward the pith, along with eight to ten purely phloem masses, more externally placed. (2) The internal phloem masses have undergone marked development, especially in the lower part of the axis. In the hypocotyl this is so pronounced that in old stems huge oval phloem areas may project into the broken pith cavity. Each of these, moreover, consists of a wide tract of most internal phloem, of a considerable tract of xylem or, instead, of two or three xylem masses separated from one another by narrow medullary rays; while the most external part of the bundle so narrows toward the protoxylem of each normal bundle that one may well speak here of complete pith bundles. It should be said, however, that for these, as for the extrafascicular bundles of *Momordica*, no spiral tracheae representing a protoxylem exist.

So far as the writer's material has been investigated, these complete pith bundles in *Cucurbita* are the largest yet observed, and deserve more careful and detailed study than has yet been given them.

Physiological inquiry

The extensive development of metaxylem in the root is correlated with the important functional work of carrying upward copious supplies of crude sap from the soil. The development of normal external phloem only in this part of the axis indicates that sufficient elaborated sap can thus be passed down and distributed to even the finest rootlets and root hairs by this tissue, unaided by auxiliary phloem tissues.

The evolution of an internal phloem primarily in the lower part of the young hypocotyl indicates that additional and abundant interchange of elaborated food must be perfectly insured from the region of the hypocotyl upward. In primitive, slow-growing cucurbits, the distribution of elaborated food evidently conforms to that seen in average shrubs and trees. With increasing rapidity of growth and increase of leaf surface, however, new methods have evolved for insuring rapid distribution of the elaborated sap.

The low branching habit of most herbaceous cucurbits, in which three to eight radiating branches start from as many originally young nodes near the ground, necessitates the development of a central correlated system of food exchange that shall reach every part of the aerial axes; hence the extremely complex intercommunications in later stages of growth of the hypocotyl and succeeding epicotylary internodes, so that a balanced distribution of crude sap supply is insured throughout the entire vascular system, upward and downward. The opinion might be ventured that the gradual development of ectocyclic phloem anastomoses may have started as a conduit system for the absorption and transfer of elaborated food gathered from the extensive chlorenchyma of the stem itself. In connection with this, the usually small and abundant (*Momordica*), as well as the few and heavy fruits (*Citrullus*), necessitate rapid and abundant passage of elaborated sap to these, no matter whether situated along a main axis or some side branch.

As in the case of previous investigators on the Cucurbitaceae, the writer has frequently referred to the marked reduction in size, or the occasional absorption of one, two, or rarely three bundles in the normal ring. It seems probable that such may be due to mechanical strains operating along the axis, and also dorsiventral light relation, for it should be remembered that while the rapidly growing cucurbits are provided with tendrils, and show many other characteristics of climbing plants, they are usually grown as scrambling vines that exhibit marked dorsiventrality after two or three weeks of growth.

The writer has been impressed by the capacity and the plasticity or adaptability shown by different areas of parenchyma for taking on new tissue formation. TAYLOR (18) demonstrated the transformation of prospective xylem cells into phloem, or vice versa, and the formation of bundle in pith cells under appropriate stimuli. Similarly, in the gradual evolution of the herbaceous, and particularly of the rapidly growing and succulent herbaceous cucurbits, it seems that any parenchyma or tissue center within the endodermis, possibly even beyond (cf. FISCHER's ectocyclic sieve tubes), is capable of modifying its future tissue history.

From the standpoint of plant mechanics, attention might now be drawn to a curious tissue change noted in several genera (*Cucurbita*, *Momordica*, *Benincasa*, and *Lagenaria*). In all of these the young roots and hypocotyl early develop strong patches of hard bast along the exterior of each bundle. These collectively seem to give a degree of tensile strength and resistance that would be eminently helpful against tugging strains exerted on the hypocotyl and root. As one approaches the cotyledonary junction, these thin out and disappear, but as the epicotyl lengthens a lignified endocortex early arises and persists for several weeks, although it gradually becomes stretched and broken through the growth expansion of the stem. Such an expanded cylinder must act as a pliable and adjustable strengthening zone in the resistance to wind strains and torsions. On the other hand, in *Momordica*, the hard bast patches of the root are continued upward through the stem, and, together with the extrafascicular bundles, provide sufficient support, and in such a case no fibrous endocortex is encountered.

Summary

1. Members of this group may form a growth of stem and leafage within the growing period of four months that is probably unequalled by any other annual herbaceous plant family. *Cucurbita Pepo* may show a stem and branch length together totaling over 140 feet, producing probably as many as 450 leaves. Plants propagated at the same time may show a surprising total growth regularity. Three specimens, differing greatly in number of branches and general external habit of growth, when measured totaled 37.25, 37, and 39 feet, and produced respectively 120, 123, and 123 leaves.

2. For a proper estimation of the nutritive activities of these plants detailed investigation must be made of the structure and growth of all parts of the plant axis, from the seedling to the mature state.

3. Study of four representative seedlings reveals in all a tetrarch bundle system in the root, each radial arm of which undergoes splitting of the protoxylem and passage of the protoxylem halves toward the metaxylem, and later uniting in front of it to constitute the four protoxylem areas of the four stem meristeles.

4. The tetrarch vascular system in the root separates into four meristeles at the base of the seedling hypocotyl. These, in their turn, start to split radially until eight or ten bundles are formed, which traverse the greater length of the hypocotyl.

5. In this transition, the four meristeles that move apart as the pith expands in the base of the hypocotyl show no trace of internal cambium. This remains true for the lowest 2-5 mm. of the hypocotyl. In their earliest seedling condition, therefore, these bundles are only collateral, in this respect resembling the more primitive shrubby cucurbits.

6. About 2-3 mm. above the summit of the root, the intrafascicular cambium of each bundle is seen to become continuous from outside inward, with a zone of fundamental cells around the lateral and anterior faces of each bundle, and thus originates what the writer designated "perixylary" cambium.

7. Through immediate activity of the inner cells of this cambium ("prefascicular" cambium), patches of internal phloem originate in front of each bundle.

8. In all of the seedlings studied, the meristeles constitute a ring of four, in time becoming eight or ten members, four or five of the ten being slightly larger and more internal, the rest slightly smaller and more external. The whole constitutes a typical dicotyledonous meristele system. There is no ground for the view that the Cucurbitaceae showed primitively multiseriate or irregularly scattered bundle disposition.

9. In transition from the hypocotyl to the epicotyl, similar details of structure and growth are usually shown. To this there is an exception, for some cucurbits develop only eight or ten strong hard bast areas, which are directly continued into the epicotyl (*Momordica*), while in most the hard bast of the radicle and hypocotyl thins out and disappears, and a lignified endocortex ring becomes the substitute layer. The writer accepts it that the early formed fibrous zone is endocortex. Within it, a one-layered endodermis and one to three-layered pericambium originate.

10. Growth of the internal phloem is effected by activity of the prefascicular cambial section, although for a time the lateral pericambial sections remain dormant. Simultaneously with the

last, sinuous interfascicular cambial patches join with the intra-fascicular to form a continuous and typically dicotyledonous cambium ring. A marked difference is observable between the small flat cambial cells of the latter and the large clear cells of the former.

11. Contemporaneous with both of these, continued division and growth of pericambial cells gradually cause stretching of the exterior part of the stem, and rupture of the fibrous endocortex where such exists.

12. Great variation is shown in the amount and degree of lignification of the developing xylem in different types, from highly parenchymatous bundles of *Citrullus* and *Cucurbita* to the dense and fibrous types such as *Luffa* and *Momordica*.

13. Tylosis cells invading the pitted vessels are observed in practically all of the genera in varying extent.

14. Crystals are very abundant and highly visible in the medullary rays and pith cells in *Momordica*.

15. The reactivated perixylary lateral cambia give rise to varying amount of lateral phloem: (1) scarcely traceable in *Momordica*, (2) slightly in *Trichosanthes*, (3) well marked as platelike patches in *Luffa*, (4) extensive in *Lagenaria*, and (5) most pronounced in *Citrullus*.

16. Such lateral medullary phloem becomes, with the addition of metaxylem, "transmedullary" bundles which attain their greatest size and variety of structure in the mature hypocotyl.

17. These masses of phloem develop from the perixylary cambial segments tangentially.

18. In the formation of these transmedullary bundles, "admedullary" rays separate the inner and larger ones.

19. Endocyclic sieve tube connections, as traced by FISCHER, become increasingly extensive with age, especially in the larger and softer types.

20. Soon after the formation of the endocyclic, ectocyclic phloem is formed by outpushing of the phloem between the burst fibrous endocortex patches, and so invasion of the mediocortex results.

21. The internal phloem areas may remain rounded and sharply demarcated as projections into the pith in the stem, but in the

lower part of the axis and in the hypocotyl of the more rank growing types these may enlarge greatly, becoming attenuate toward the protoxylem front, and eventually become occluded into the pith, after developing centrifugal xylem, as "obcollateral" bundles.

22. More or less throughout the older axis, and especially in the hypocotyl of *Cucurbita Pepo* and *Citrullus vulgaris*, "intraxylary" phloem in the xylem parenchyma appears.

23. An extensive system of secondary medullary rays arises in all types, the manner and depth of these corresponding to the thickness of the stem.

24. In species of *Momordica* alone a unique formation of "extrafascicular" bundles is observed, and these do not develop simultaneously and are not of equal size, but in succession over the stem. They extend from the hypocotyl only through a limited number of nodes of the epicotyl, to disappear above.

25. The xylem and phloem bridge connections link all the meristeles together, especially in the hypocotyl.

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EXPLANATION OF PLATES I-III

Abbreviations: *cex*, cambium of extrafascicular bundle; *ec*, external or intrafascicular cambium; *ecst*, ectocyclic sieve tubes; *enc*, endocortex; *enst*, entocyclic sieve tubes; *eph*, external phloem; *hb*, hard bast; *hber*, hard bast of extrafascicular bundle; *ic*, internal or prefascicular cambium; *ifc*, interfascicular cambium; *iph*, internal phloem; *ixph*, intraxylary phloem; *mc*, medullary (ray) cambium; *mph*, medullary phloem; *p*, pith; *phco*, phloem bridge connection; *pmr*, primary medullary ray; *pmrex*, primary medullary ray of extrafascicular bundle; *pv*, pitted vessel; *rph*, rudimentary phloem; *sb*, soft bast; *sber*, soft bast of extrafascicular bundle; *smr*, secondary medullary ray; *smrex*, secondary medullary ray of extrafascicular bundle; *sv*, spiral vessel; *tmr*, tertiary medullary ray; *xco*, xylem bridge connection; *xex*, xylem of extrafascicular bundle; *xp*, xylem parenchyma.

PLATE I

FIG. 1.—Diagrammatic transverse section of seedling root of *Luffa aegyptiaca*, showing typical tetrarch monostele; $\times 90$.

FIG. 2.—Diagrammatic transverse section of seedling root of *Luffa aegyptiaca*: monostele splitting into four meristeles, two xylem masses larger, two smaller; note development of side root in line with a xylem arm; $\times 90$.

FIGS. 3-6.—Stages in separation of root monostele into four stem meristeles: $\times 150$.

PLATE II

FIG. 7.—One of meristeles represented in fig. 6, showing development inward of perixylary cambium forming internal or prefascicular cambium; note spiral tracheae at both extremities of bundle; $\times 250$.

FIG. 8.—Same, showing further development of internal or prefascicular cambium, here given rise to small patch of internal phloem; note moving inward of spiral tracheae to position immediately internal to pitted vessels; $\times 250$.

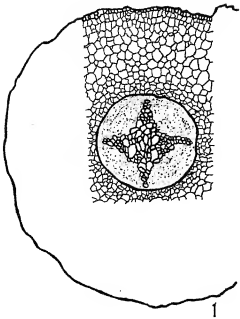
FIG. 9.—Transverse section of meristele of *Luffa aegyptiaca* in lower hypocotyl just above level of "heel," showing perixylary cambium and newly developed internal phloem; $\times 160$.

FIGS. 10, 11.—Transition stages from root to hypocotyl in seedling of *Momordica balsamina*; note hard bast already developed; $\times 115$.

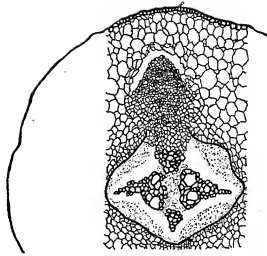
FIG. 12.—Transverse section of meristele in lowest part of seedling hypocotyl of *Momordica balsamina*, showing prominent hard bast; $\times 115$.

PLATE III

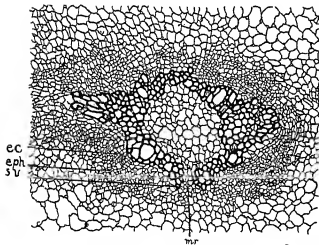
FIG. 13.—Diagrammatic transverse section of mature stem of *Trichosanthes colubrina*, fifth internode from apex, showing broken sclerenchyma ring (*enc*), rudimentary phloem areas (*rph*) of two inner bundles; note commencing medullary phloem (*mph*) (interfascicular cambium not shown); $\times 15$.



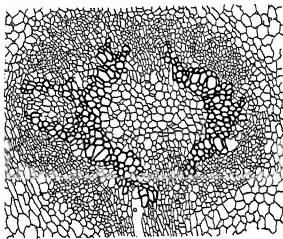
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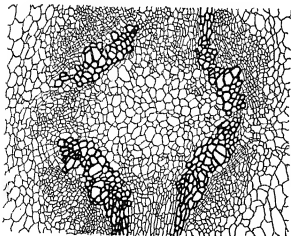
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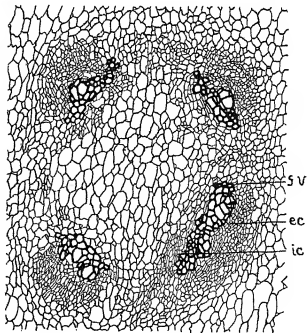
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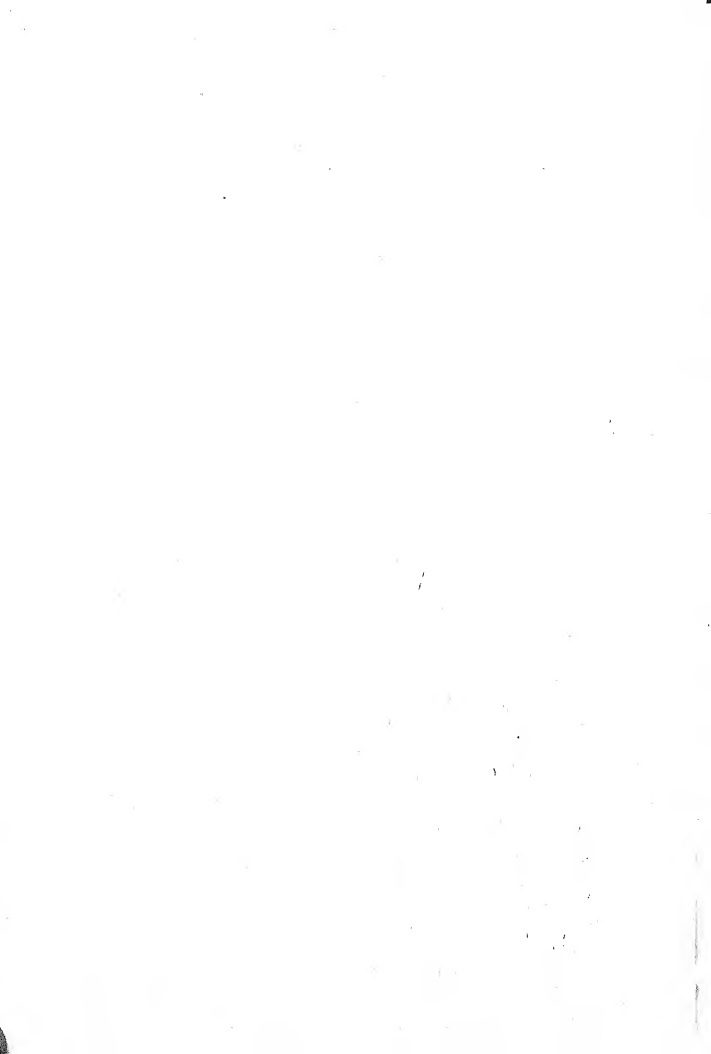


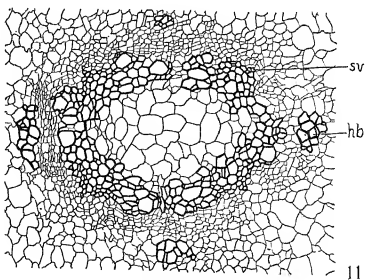
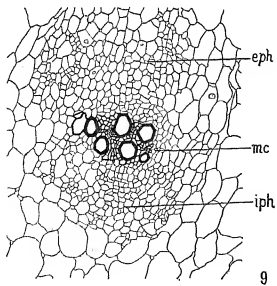
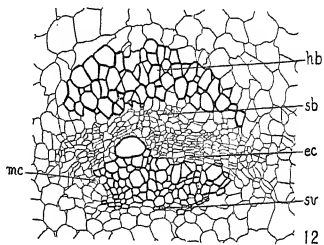
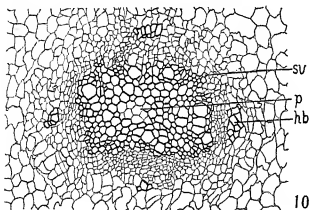
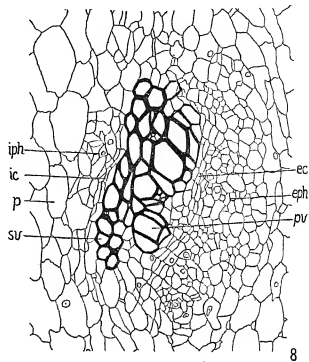
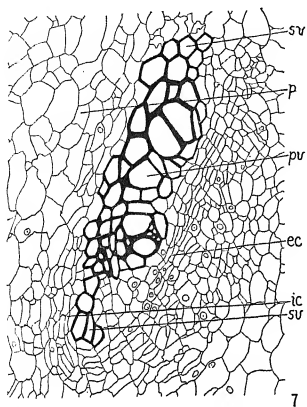
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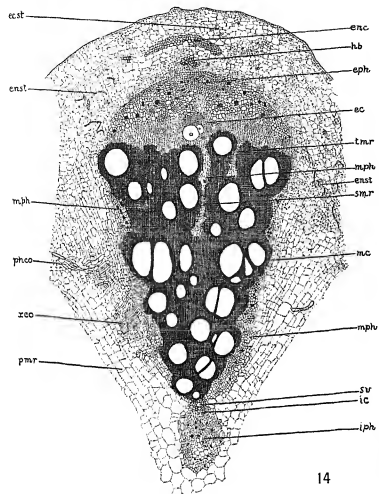
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HOLROYD on CUCURBITACEAE

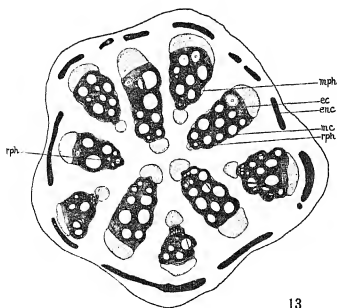




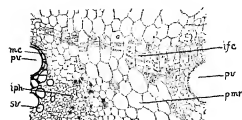




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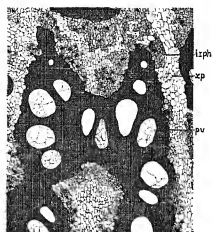
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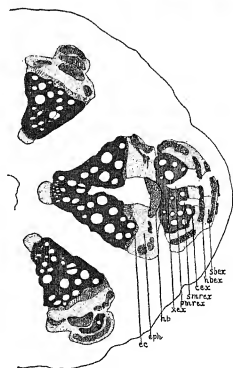
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16



17



18



FIG. 14.—Same, first epicotylary internode above hypocotyl, showing medullary phloem in primary and secondary medullary rays (*mph*), xylem and phloem bridge connections (*xco*, *phco*), ectocyclic and endocyclic sieve tubes (*ecst*, *enst*); (in part diagrammatic); $\times 25$.

FIG. 15.—Transverse section of medullary ray of *Luffa aegyptiaca*, showing conspicuous interfascicular cambium in stages of development; $\times 32$.

FIG. 16.—Transverse section of mature hypocotyl of *Luffa aegyptiaca*, showing section of primary medullary ray with medullary phloem and part of perixylary cambium extending alongside metaxylem; $\times 32$.

FIG. 17.—Transverse section of xylem area of mature hypocotyl of *Citrullus vulgaris* at end of growing season, showing intraxylary phloem in xylem parenchyma; note tylosis cells in pitted vessels; (in part diagrammatic); $\times 25$.

FIG. 18.—Diagrammatic transverse section of mature hypocotyl of *Momordica balsamina*, showing details of extrafascicular bundles adjacent to each normal bundle; note persistent hard bast of normal bundles, one to three alternating layers of hard and soft bast of extrafascicular bundles, absence of medullary and intraxylary phloem, and absence of sclerenchymatous endocortex; $\times 15$.

DIVERGENCE OF CATALASE AND RESPIRATION IN GERMINATION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 320

LOUISA E. RHINE

(WITH FOUR FIGURES)

Introduction

Interest in catalase centers around two main points: (1) its nature and function, and (2) its use as a measure of respiratory or metabolic activity. Little is known of its nature except that it acts like an enzyme in hastening a reaction that would proceed very slowly without it. Hydrogen peroxide decomposes spontaneously, but very slowly. The single known property of catalase is its power to hasten greatly this reaction. It is further like an enzyme in its temperature relations (24, 29, 34, 40), and in the general methods by which it may be extracted and precipitated. Although such facts as these have led more and more to the general belief that catalase is an enzyme, as the earlier workers (25, 29, 37) thought, it is for convenience rather than certainty that it is so spoken of here. Although the knowledge of the nature of catalase is far from complete, its function in the cell is still more of a mystery. By far the majority of workers in this line have found some connection between catalase and oxidation or respiration, but the data reported offer too many contradictions to permit of any conclusions regarding the nature of this connection. Among the various theories advanced regarding the possible function of catalase in the cell is that of BURGE and his co-workers (8, 10, 11, 12, 13, 14). This interprets catalase as having a causal relation to oxidation, apparently because catalase activity and oxidation rate parallel each other in many cases, from which the conclusion is drawn that the one is the cause of the other. This theory might be taken more seriously if it were the only possible explanation of the facts, and if there were not so many instances in which catalase activity and

respiration are not parallel. Even though in many cases a high metabolic condition in tissue is accompanied by a high catalase content, the accumulation of evidence showing discrepancies in the two processes (1, 8, 33, 39, 41, 44, 46) should be taken as indication that this parallelism may be incidental rather than fundamental.

Although numerous other theories of the function of catalase have been advanced (6, 7, 20, 21, 25, 45), that of LOEW (29) has perhaps been most widely accepted (23, 30, 38). This rests on the supposition that hydrogen peroxide is formed in the process of oxidation, and that catalase functions to protect the cell against toxic concentrations of it by decomposing it at once into harmless constituents. The chief objection has been that hydrogen peroxide has never yet been demonstrated as actually occurring in cell oxidations, although it seems not improbable that it is so formed (17). Besides this, catalase apparently will not react with other peroxides (5). As LOEW (30) points out, however, the fact that the presence of hydrogen peroxide has not been proved is not so great an objection to the theory as it might seem, since even if it were formed in a cell containing catalase it would necessarily be decomposed almost instantaneously and before it could be detected experimentally. Besides this there is the other supposition, that, although no other substance except hydrogen peroxide has been found on which catalase will act *in vitro*, it is quite possible that it functions in the cell as LOEW suggested, but upon an unknown substance which may equally well be formed in the process of oxidation.

The fact that in so many cases (2, 3, 4, 10, 12, 13, 14, 16, 19, 28, 31, 46, 47) a parallelism has been shown between catalase and respiration has led in some degree to the use of catalase as a measure of oxidation rate. If it could be demonstrated that this is a safe procedure, it would certainly offer a convenient substitute for the tedious and too often impossible direct measurement of respiration and metabolic rate. The discrepancies mentioned, however, indicate that more must be known of the nature of catalase and its place in metabolism before it can be decided how far its use as an indicator is safe and valid. In the paper here presented it is hoped to shed some light upon this problem.

Method of catalase determinations

The method used was essentially that described by APPLEMAN (2), in which the oxygen liberated is measured by a manometer with adjustable arm, so that excess pressure during the reaction is avoided. The water bath was kept between 21° and 22° C. and all results calculated to standard conditions of temperature and pressure. Five cc. of Oakland "dioxogen" neutralized with N/10 NaOH was used in all cases. The tissue was ground in a mortar with a small quantity of sand and an excess of CaCO₃. It was washed quantitatively into the shaking bottle with about 15 cc. of distilled water. When it had reached the temperature of the bath, the hydrogen peroxide was added 15 seconds before the shaking began. One 10-minute reading was taken.

In using various kinds of seeds in different conditions of moisture content, it was found impossible to adjust the degree of pulverization of tissue by any arbitrary method. Since the determinations were to be quantitative, a sieve could not be used, and it was equally impossible to choose a single length of time of grinding for all seeds, owing to the great diversity of hardness, moisture content, and general character of the tissue. The material was ground, therefore, to what in best judgment seemed to be a comparable state of fineness and homogeneity with that attained in grinding dry wheat four minutes.

MORGULIS and others (9, 32, 33) object that a time reading, arbitrarily chosen for the measurement of the catalase-hydrogen peroxide reaction, cannot afford an accurate measurement of catalase activity. Under the law of mass action, an arbitrary time limit would not express the results of comparable stages in the reaction when the amount of one or more substances was varied. Thus when the proportion of catalase to hydrogen peroxide is high, a 10-minute reading would express the results of a much greater part of the entire reaction than it would when the amount of catalase is relatively small. The data in table I will make this clear. The reactions were allowed to run to completion, one, two, five, ten minute and final readings made, and the time until the end of the reaction recorded. Dry wheat was the tissue used.

It is apparent from table I that a 10-minute reading does not give a measure of the maximum amount of oxygen delivery in any but one of the tests made. Five cc. of "dioxogen" is capable of yielding 67.8 cc. of oxygen, as measured by the maximum amount secured with an excess of catalase-containing tissue. In every case except the first two, therefore, the amount of catalase present was in excess of the peroxide. The greater this excess, the shorter is the time required to complete the reaction.

MORGULIS (32) has shown further that the presence of a great excess of either hydrogen peroxide or catalase exerts a depressing influence on the reaction. This is shown in table I in every case beyond the second. MORGULIS criticizes the "entire method of

TABLE I

NO. OF GRAINS	WEIGHT IN GM.	O ₂ (CC.) LIBERATED IN				FINAL AMOUNT OF O ₂ (CC.)	TOTAL TIME IN MINUTES
		1 minute	2 minutes	5 minutes	10 minutes		
5.....	0.185	3	5	8	12	27.3	120
10.....	0.385	6	11	18	26	60.8	105
15.....	0.601	10	16	30	40	67.8	105
25.....	0.933	12	20	33	45	67.1	55
50.....	1.844	22	34	51	56
75.....	2.896	23	37	57	66	67.8	25
150.....	5.2	30	46	63	67	67.2	14

comparing several samples of catalase on the basis of the amount of oxygen which they respectively liberate," and states that the comparison should rather be "between respective quantities of catalase preparation required to set free the same amount of oxygen from a given quantity of peroxide." This change in method, however, would involve a great loss of time in determining the correct amount of catalase to be used at each determination, and is unnecessary because the whole error involved in the old method can be corrected for with comparative ease, by a simple set of factors designed to apply within a wide range of possible oxygen delivery. The following set has been planned to apply to the kind of material and reaction that has been used in the following experiments. Dry wheat was pulverized, sifted through a 100-mesh screen, and thoroughly mixed to give a flour of the greatest possible homo-

gency. Catalase determinations were then made on amounts of flour varying from 0.02 to 2 gm., at close intervals. A 10-minute reading was taken, and from this and the weight of material used the catalase activity per gram was calculated. Table II shows the results and the factors calculated from the maximum value per gram.

These results indicate that when the amount of catalase present during a determination is so adjusted that it liberates about 19 cc. of oxygen in 10 minutes, the maximum value is being obtained. Thus it follows that when using 5 cc. of full strength neutralized "dioxogen" at 21°-22° C. with pulverized seed material, results

TABLE II

Weight in gm.	O ₂ (cc.) liberated in 10 minutes	O ₂ (cc.) per gm.	Factor
0.02.....	0.491	24.55	1.34
0.04.....	1.01	25.55	1.30
0.06.....	1.56	26.00	1.26
0.08.....	2.06	25.76	1.28
0.1.....	3.01	30.10	1.07
0.2.....	6.38	31.94	1.03
0.4.....	12.92	32.30	1.02
0.6.....	19.77	32.95	1.00
0.8.....	24.55	30.69	1.06
1.0.....	30.80	30.80	1.07
1.2.....	35.82	29.84	1.13
1.4.....	39.49	28.20	1.14
1.6.....	42.02	26.26	1.25
1.8.....	44.49	24.71	1.33
2.0.....	47.82	23.91	1.37

will be true when the oxygen liberated in 10 minutes is about 18-20 cc. Results on either side of this figure can be corrected for by using the factors given in column four. This set of factors, however, cannot be used to give accurate correction for all types of tissue that might be investigated, for it seems evident that the bulk of material present in the comparatively inert dry seed meal exerts some influence on the course of the reaction. Table III shows data similar to those given in table II, but in this case, instead of the wheat flour, a comparatively active water extract of young rye ovules was used.

Fig. 1 shows the data presented in tables II and III in the form of curves. While the same general type of curve is secured in each

instance, there is a distinct difference in the rate of depression and in the position of the highest value in each curve. Since the material used in all of the experimental work which follows was of the ground seed type represented in table II and curve I of fig. 1 the corrections indicated in table II have been used throughout.

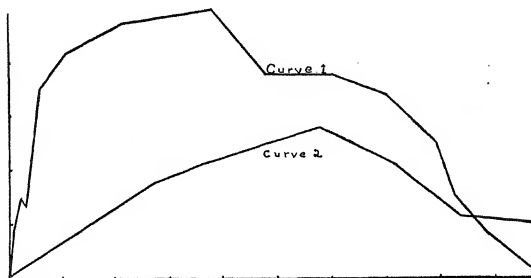


FIG. 1.—Curves showing variation in type of reaction of two kinds of catalase-containing tissue: horizontal axes represent cc. of O_2 delivery in 10 minutes, vertical axes represent cc. of O_2 delivery per unit; curve I, from data in table II (O_2 from 1 gm. taken as unit); curve II, from table III (O_2 from 3 cc. taken as unit).

TABLE III

Extract (cc.)	O_2 (cc.) liberated in 10 minutes	O_2 (cc.) per cc.	Factor
0.5.....	4.0	8.0	1.18
1.0.....	8.65	8.65	1.09
1.5.....	13.32	8.96	1.05
2.0.....	18.66	9.33	1.01
3.0.....	28.43	9.47	1.00
4.0.....	36.26	9.05	1.04
5.0.....	43.48	8.60	1.10
6.0.....	50.15	8.35	1.14

It may be noted in passing, however, that in no case have the corrections involved been sufficiently large to change the nature of the result in a radical degree. An attempt was always made to adjust proportions somewhat to secure results ranging as closely as

possible around 18-20 cc. of oxygen delivery. Since the great individual variation in seeds makes it impossible to secure perfect checks, all determinations were run in triplicate, and the numbers reported are the averages of these determinations.

All the seeds used were obtained from Vaughan and Company, Chicago, and were of the 1922 crop. Only those showing a germination of 95 per cent or better were used.

TABLE IV
WHEAT, 10 SEEDS PER TEST, WEIGHT 0.385 GM.

Time	Condition	Uncorrected O ₂ (cc.)	Factor	Corrected O ₂ (cc.)
A. Germination temperature 10°-12° C.				
Days				
0.....	Dry	21.50	1.00	21.5
1.....	Germination	11.53	1.02	11.76
2.....	Germination	10.15	1.02	10.36
3.....	Germination	10.16	1.02	10.36
4.....	Germination	10.43	1.02	10.64
5.....	Germination	11.09	1.02	11.31
6.....	Germination	12.64	1.02	12.89
7.....	Germination	17.47	1.00	17.47
8.....	Germination	17.80	1.00	17.80
B. Germination temperature 23°-26° C.				
Hours.....				
.....	Dry	21.50	1.00	21.50
4.....	Germination	13.98	1.02	14.25
11.....	Germination	12.75	1.02	13.00
15.....	Germination	14.47	1.02	14.75
24.....	Germination	20.77	1.00	20.77
41.....	Germination	36.05	1.13	40.74

Investigation

A study of the relationship between catalase activity and respiration rate under conditions of rapid change was undertaken in the hope that it would throw some light upon the nature of catalase and its relation to oxidation. Germination and ripening of seeds seemed to offer examples of such change. These phases were therefore chosen for detailed study.

CATALASE IN GERMINATING SEEDS.—Tables IV-IX show the results of catalase determinations made upon six different kinds of seeds at various stages in their germination. For convenience of

determination, and to avoid somewhat the error introduced from individual variations in seeds, small seed types were chosen, and ten or more seeds used in each test according to their relative

TABLE V

FETERITA, 10 SEEDS, WEIGHT
0.332 GM. 23°-26° C.

Hours	Condition	Corrected O ₂ (cc.)
0.....	Dry	13.0
2.....	Germination	8.14
7.....	Germination	6.19
14.....	Germination	6.52
21.....	Germination	7.19
30.....	Germination	9.5
40.....	Germination	10.06
48.....	Germination	16.69

TABLE VI

CLOVER, 10 SEEDS, WEIGHT
0.007 GM. 23°-26° C.

Hours	Condition	Corrected O ₂ (cc.)
0.....	Germination	18.3
2.....	Germination	12.77
8.....	Germination	8.79
22.....	Germination	8.63
28.....	Germination	4.09
48.....	Germination	10.56

TABLE VII

MUSTARD, 280 SEEDS, WEIGHT
2 GM., 25° C.

Hours	Condition	Corrected O ₂ (cc.)
0.....	Dry	3.56
5.....	Germination	2.88
11.....	Germination	2.96
24.....	Germination	12.15
33.....	Germination	14.19

TABLE VIII

RADISH, 25 SEEDS, WEIGHT
0.2 GM., 25° C.

Hours	Condition	Corrected O ₂ (cc.)
0.....	Dry	26.36
0.5.....	Germination	21.36
1.0.....	Germination	20.75
2.0.....	Germination	24.89
3.0.....	Germination	31.83

TABLE IX

BUCKWHEAT, 20 SEEDS, WEIGHT
0.515 GM., 25° C.

Hours	Condition	Corrected O ₂ (cc.)
0.....	Dry	13.15
4.....	Germination	8.15
10.....	Germination	5.87
18.....	Germination	4.79
27.....	Germination	15.07
34.....	Germination	26.02

activity. The six kinds of seeds studied were selected further to include starchy, fatty, and protein types, but beyond that were chosen at random.

Tables IV-IX and fig. 2 show that at the very outset of germination there is a decided and unmistakable drop in the amount of catalase per seed. In each of the six different kinds of seeds tested the same striking decrease is shown. It begins apparently as soon as the seed is put in germinating conditions, and the period of its duration is proportionate to the rate of germination. The extreme of rapidity of this initial drop is interestingly shown in the data on radish in table VIII. Here in one-half to one hour the lowest point is reached, and in three hours the seed is practically germinated, the testa being ruptured and the cotyledons protruding. In

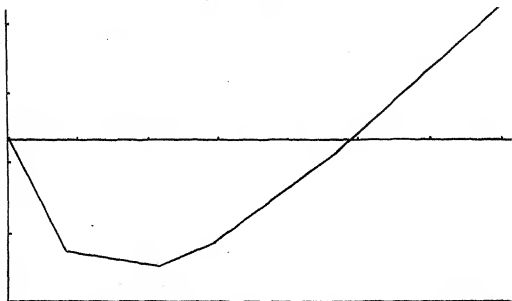


FIG. 2.—Typical catalase germination curve, plotted from data in table IV A: horizontal axes represent time of germination in hours; vertical axes represent cc. of O₂ delivered in 10 minutes.

other cases the depression lasts longer. In wheat at 10°-12° C. (table IV A) the lowest point recorded is not reached for several days, while in the same seed at 23°-26° C. it is reached in half a day. This difference, however, is quite in proportion to the time required for the germination of wheat at these two temperatures.

This decrease in catalase is quickly followed by a sharp rise, such as has ordinarily been reported for catalase during germination (18, 26, 36). The very early stage at which the drop occurs is doubtless why it has been overlooked in previous work, since by the time the hypocotyl is protruded, in most of the seeds tested, the

catalase activity is back again to about the amount that had been present in the dry seed.

CATALASE IN WET SEEDS DEPRIVED OF OXYGEN.—Coincident with the study of the germination of seeds, the effect of prolonged soaking in water was tested.

The data on soaking seeds confirm those secured on the germinating ones. The same initial decrease found in that case is present here also. In this case, however, the curve continues to go down, with no subsequent rise such as was shown in the data on germination. The seeds used in table IV A and table X A were kept side by side on ice during the same period, so that the two sets can well

TABLE X
WHEAT, 10 SEEDS PER TEST

Days	A. 10°-12° C.		Days	B. 13°-16° C.	
	Condition	Corrected O ₂		Condition	Corrected O ₂
0...	Dry	21.5	0...	Dry	17.17
1...	Soaking in tap water	15.66	1...	Soaking in boiled distilled H ₂ O	14.95
2...	Soaking in tap water	15.88	2...	Soaking in boiled distilled H ₂ O	14.31
3...	Soaking in tap water	11.28	3...	Soaking in boiled distilled H ₂ O	12.46
4...	Soaking in tap water	12.02	10...	Soaking in boiled distilled H ₂ O	8.89
5...	Soaking in tap water	10.52	17...	Soaking in boiled distilled H ₂ O	7.31
7...	Soaking in tap water	11.20	22...	Soaking in boiled distilled H ₂ O	0.6
8...	Soaking in tap water	11.02	29...	Soaking in boiled distilled H ₂ O	0.5
15...	Soaking in tap water	10.98	22...	Germination 2 per cent
19...	Soaking in tap water	10.44	29...	Germination none

be compared. There is significance in the fact that the germinating seeds show a more rapid decrease at first than the soaking ones. This will be discussed later. This experiment was interrupted by accident, but was repeated later in table X B. In this case, however, a somewhat higher temperature, and boiled, distilled water (in place of the not entirely oxygen-free tap water) was used, and the time for catalase exhaustion reduced to 22-29 days. At the end of 22 days the seeds were tested for viability, and a feeble germination of about 2 per cent was secured. In 29 days none germinated. A decrease in catalase activity with soaking of seeds has been noted incidentally in earlier work (35, 41, 42), but its significance was not recognized.

DRY SEEDS DEPRIVED OF OXYGEN.—Since catalase was seen to decrease to practical exhaustion under oxygen-free conditions in the presence of water, an attempt was made to produce the same result on dry seeds. Wheat grains were imbedded in soft paraffin to exclude the air, and tested again after 65 days. The catalase activity was still practically unchanged, however, and it was evident that changes in the dry seeds resulting from lack of oxygen occur at such a slow rate that a much longer time would have to elapse before they would be measurable.

DISTRIBUTION OF CATALASE IN SEED DURING GERMINATION.—In order to learn something of the distribution of catalase in different parts of the seed in various stages of germination, a series of tests was made. Dry seeds could not be separated into endosperm

TABLE XI
WHEAT, 10 SEEDS WHOLE, ENDOSPERM OR EMBRYO, PER TEST

No. of test	Condition	O ₂ whole seed (cc.)	O ₂ endosperm (cc.)	O ₂ embryo (cc.)
1.....	Dry	21.5
2.....	Soaked 1 hour	17.00	13.0	6.0
3.....	Germination, 2 days 10°-12° C.	10.15	6.7	3.7
4.....	Germination, 7 days 10°-12° C. (just sprouted)	17.49	7.0	8.0
5.....	Germination, 41 hours 25° C. (well sprouted)	36.00	15.0	16.0
6.....	Germination 3 days 25° C. (seedlings)	19.0

and embryo with scutellum, and, therefore, seeds soaked for one hour (table XI, no. 2) had to be used instead to give the closest possible indication of the distribution of catalase in a dry seed. No. 3 of table XI shows the distribution of catalase between embryo and endosperm when the activity of the entire seed is at its lowest ebb in germination. Nos. 4-6 show the distribution as the activity of the whole seed rises. The pertinent facts brought out by table XI are: (1) that both the endosperm and the embryo show a drop in catalase paralleling that in the whole seed; and (2) that the endosperm as well as the embryo shows a later increase. Of course it does not rise as high in the endosperm as in the enlarging embryo, but no. 6 shows it to be higher again than it was in no. 2, at least,

although by this time it appears quite dry and shriveled. It must be remembered, however, that the layer of aleurone cells with their living protoplasm is included in all the tests on endosperm.

RESPIRATION OF SEEDS DURING GERMINATION.—In the past catalase and respiration during germination have always been reported as paralleling each other, and increasing steadily (15, 18, 26, 36, 41, 46). Since, however, it is shown that catalase activity decreases in early germination, it becomes important to know what course respiration takes during this period. Either respiration must also suffer a decrease with the onset of germination, or, as has been supposed, it increases steadily from the beginning of germination. If the latter should be the case, we would have here a striking divergence in the two curves. In the literature there are many instances of small differences in the rate or intensity of the two processes, but since apparently there is no case on record showing decrease of catalase activity with increase of respiratory rate, it was thought worth while to make a careful study of the respiration, at short intervals, of germinating seeds throughout the period of catalase decline.

Since no convenient method was available for measuring increase of respiration consecutively on the same seed sample at short intervals, a simple apparatus was contrived by which such data could easily and quickly be secured. The principle involved was the direct measurement of changes in air volume arising from respiration when the carbon dioxide is absorbed by NaOH. By this method a seed sample could be used that was sufficiently large to overcome the error arising from the individual variations of the seeds, and readings could be taken at will on the same seeds throughout the germination period. An outline of the apparatus is shown in fig. 3.

Two instruments thus fitted can be used, one containing the seeds and serving as the test, the other, with everything but the seeds, being a control on temperature and pressure conditions. This obviates the necessity of making calculations for the corrections necessary for these variables, which would have to be made if one instrument were used alone. The temperature of the two vessels must always be kept the same, however, since in air volumes

of this size, even a temperature fluctuation of 0.05 of a degree causes a readable difference in volume. When this temperature control can be obtained, the readings are made very simple, for the rise in the manometer of the test over that of the control measures directly the amount of oxygen consumed by the seeds. The CO_2 may be determined by titration of the NaOH , or by the use of a third desiccator in which seeds but no CO_2 -absorbent are placed.

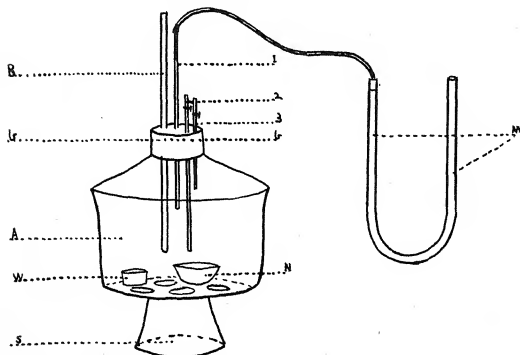


FIG. 3.—Respiration apparatus: *A*, large desiccator with stoppered lid, seeds to be tested placed at *S*; *W*, small container for water to keep air saturated; *N*, dish with wide surface for NaOH ; rubber stopper at top is fitted with Beckman thermometer *B* and three small glass tubes 1, 2, and 3; tube 1 connects interior of desiccator with loose arm manometer *M*, tube 3 is exit for escape of air when NaOH is added at tube 2, which with stopcock serves for introduction of NaOH to dish *N* below; desiccator immersed in constant temperature water bath to line *b*, and NaOH added from burette after desiccator is closed and in place, to protect from CO_2 of air as much as possible.

If this is under the same temperature control as the other two, the O_2 and CO_2 difference can be read directly from this manometer. Then, knowing the value of the O_2 , the CO_2 may easily be determined, and the ratio CO_2/O_2 obtained if desired. For the purposes of this experiment, however, the respiration ratio was not of interest, and therefore only two chambers were used, one for a control and one for the determination of the rate of oxygen absorption. These were immersed in a Freas water bath set at 25°C .

The fluctuation in temperature between the two, as shown by the Beckman thermometers, was negligible. Twenty-five cc. of 4N NaOH was used in both vessels, and about 30 cc. of water was kept in the water dish at all times. The amount of oxygen consumed by the dry seeds was found to be too small to be read hourly on the

TABLE XII
RESPIRATION OF GERMINATING WHEAT SEEDS, 65 GM. OF DRY SEEDS USED

Hours	Condition	Reading of CO ₂ (cc.)	Hours	Condition	Reading of CO ₂ (cc.)
48.....	Dry	2.0	8.....	Germination	4.65
1.....	Germination	1.1	9.....	Germination	5.3
2.....	Germination	1.9	10.....	Germination	5.4
3.....	Germination	2.1	11.....	Germination	7.0
4.....	Germination	3.2	12.....	Germination	7.9
5.....	Germination	3.6	13.....	Germination	10.1
6.....	Germination	4.1	14.....	Germination	17.8
7.....	Germination	4.0			

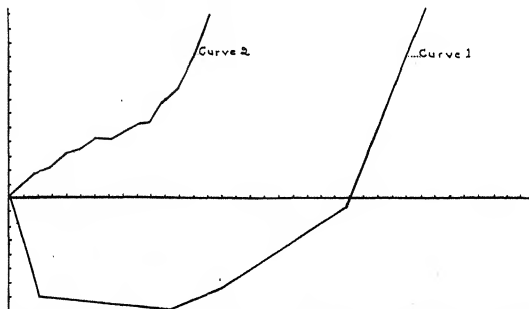


FIG. 4.—Comparison of typical catalase and germination curves during first 15 hours of germination of wheat at 23°-25° C.: curve 1, catalase, plotted from data in table IV B; curve 2, respiration, plotted from data in table XII.

manometer. The seeds were therefore left in the chamber with the absorbent for 48 hours, and the NaOH then titrated with N/10 HCl to determine the amount of CO₂ absorbed. The value from the control vessel was subtracted from the test, and the result calculated to cc. of CO₂ equivalent, at standard temperature and pressure. Table XII shows the results.

These data indicate strikingly that respiration increases tremendously from the first hour of germination. At no time is there any tendency for the respiration rate to decrease, or fall below that of the dry seeds. This shows that in early germination, catalase and respiration are not only not parallel, but are for a time running counter to each other, one decreasing while the other increases.

CATALASE IN DEVELOPING OVULES.—Since this unsuspected drop in catalase occurs in germinating seeds, it was thought worth while

TABLE XIII
RESPIRATION OF GERMINATING CLOVER SEEDS,
50 GM. PER TEST

Hours	Condition	Reading of CO ₂ (cc.)
48.....	Dry	1.2
1.....	Germination	2.1
2.....	Germination	9.5
3.....	Germination	16.5
4.....	Germination	Too large to measure

TABLE XIV
GOLDEN WAX BEAN

No.	Condition	Wet weight per ovule	Dry weight per ovule	O ₂ per gm. wet weight	O ₂ per gm. dry weight	O ₂ per ovule
1.....	Early green	0.0115	0.00167	150.9	8987.5	1.74
2.....	Early yellow	0.082	0.0095	113.0	7919.8	75.4
3.....	Late yellow	0.826	0.4910	80.6	328.5	161.2
4.....	Ripe, dry pod	0.82	0.6620	57.8	175.5	47.8

to survey the general trend of the curve of catalase activity in ripening seeds as well. Determinations were made on rye and wheat seeds throughout the period beginning just before anthesis and up to maturity, and also on wax beans from the earliest stage after blossoming that the ovules were large enough to be handled, until the pod was ripe and dry. In these tests the weight and number of seeds used in an individual test had to be shifted considerably from time to time, to bring the O₂ delivery within a range of 15-30 cc. Results were corrected and computed per gram of wet weight, dry weight, and per ovule.

Catalase in developing seeds is here shown to decrease abruptly per dry weight as the ovule develops, and to decrease also per wet weight, but less abruptly during early stages, with a more marked drop during the last few days when the moisture content is decreasing so rapidly. The data per ovule show an increase up to a central point, after which there is a rapid decrease.

TABLE XV
SPRING WHEAT

No.	Condition	Age in days	Wet weight per ovule	Dry weight per ovule	O ₂ per gm. wet weight	O ₂ per gm. dry weight	O ₂ per ovule
1.....	Before anthesis	— 2	0.0016	0.00028	161.3	8965.0	0.258
2.....	Anthesis	1	0.0042	0.0010	162.3	6763.0	0.650
3.....	After anthesis	4	0.0148	0.0043	217.0	958.4	3.28
4.....	Early green	8	0.0238	0.0055	197.3	858.0	4.93
5.....	Later green	13	0.0390	0.0110	212.0	649.2	8.05
6.....	Slightly yellow	15	0.0460	0.0220	41.0	131.0	5.30
7.....	Yellow	23	0.0350	0.0210	71.0	117.8	2.50

TABLE XVI
RYE

No.	Condition	Age in days	Wet weight per ovule	Dry weight per ovule	O ₂ per gm. wet weight	O ₂ per gm. dry weight	O ₂ per ovule
1.....	Before anthesis	— 3	0.0015	0.00022	200.9	1339.3	0.3
2.....	Anthesis	1	0.0028	0.00046	154.76	930.6	0.44
3.....	After anthesis	6	0.0109	0.00228	186.5	888.0	2.03
4.....	Early green	10	0.0274	0.00359	229.0	907.3	6.00
5.....	Later green	24	0.0400	0.00820	227.2	1108.3	9.09
6.....	Full grown	30	0.0493	0.00278	118.5	209.9	5.85
7.....	Slightly yellow	37	0.0450	0.00223	82.73	166.1	3.14
8.....	Yellow, dry	43	0.0300	0.02500	92.2	121.2	2.65

Discussion

There have been as yet no actual data to prove that catalase does have a function and is not a mere useless by-product of cell activity. LOEW's (29) idea of the protective function of catalase had nothing but a certain amount of probability to support it. In the data here presented, however, there seem to be indications that catalase must have an actual function in the cell. It is certainly used up in cell activity. When growth processes increase suddenly, the reserve catalase from the dry seed is acted upon and greatly

decreased before the mechanism that produces the enzyme is able to get into a comparable rate of activity. If hydrogen peroxide be formed in the cell and acted upon by catalase, as LOEW suggested, it is conceivable that in the sudden increase of respiration with germination, a rapid production of hydrogen peroxide might result, and the reserve catalase be quickly decreased, just as was observed.

It is shown by these results that catalase cannot be considered as part of the respiration mechanism, since one can decrease while the other increases. If catalase were directly produced as a by-product in the process of respiration, the two curves would have to parallel each other closely, and could not show the radical departure shown in these experiments. If it were instrumental in initiating respiration, or were part of it, it would not decrease in the absence of oxygen, as it is shown to do in table X, during prolonged soaking when aerobic respiration is almost entirely cut off, nor could the respiration curve continue to rise while the catalase curve falls.

The fact that it does decrease to practical exhaustion during prolonged soaking in oxygen-free water, however, shows that catalase must be at least indirectly related to the presence of oxygen and oxidation. It is rapidly destroyed in the early growth changes that occur, both in the germinating and the soaking seeds. In the latter case, however, under anaerobic conditions the normal course of the germination process is soon retarded. The seeds do not fully germinate, although a few succeed in breaking the testa slightly. The germination process is therefore completely inhibited by the oxygen starvation. This inhibitory effect might explain the fact that the decrease of catalase at first is slightly less rapid in the soaked seeds than in the germinating ones (tables IV A, and X A). In the case of the soaked seeds the basic life processes, comparable with those that go on so very slowly in the dry seed, continue throughout the following weeks, during which the seed remains viable, but is living by anaerobic respiration only. During this period the catalase continues to be used up (table X), and when this reserve is practically exhausted the germination tests show that viability also is practically gone. When oxygen is present for aerobic respiration, as in germination, a new supply of catalase is soon produced and the curve rises again, but if it is absent no

new supply forms and the reserve is in time depleted. From this it would seem that the amount of catalase depends distinctly, although indirectly, upon aerobic respiration.

From the experimental work here presented, the most plausible theory of catalase formation in the plant seems to be that it is formed as an enzyme according to the theory of need, the presence of the substance which it attacks acting as a stimulus for the production of the enzyme. In this case the substance it attacks would be, according to LOEW's hypothesis, hydrogen peroxide, or if not that, some other substance as yet undiscovered, which can react with catalase, and which is produced in the process of aerobic respiration.

Under this hypothesis the decrease of catalase with germination could be explained by supposing the catalase reserve to be used up, in attacking the respiration products, faster than it is produced. The high stimulation of this suddenly increased quantity of hydrogen peroxide (or other similarly behaving substance) would then lead in a short time to the increased production of catalase observed, just as in the ripening seed the falling off of the rate of metabolism would decrease the stimulation of the catalase producing mechanism, with a resulting decrease in catalase (tables XIV, XV, and XVI). The almost complete exhaustion of catalase in the soaked seeds then would be attributed to the using up of the reserve under anaerobic conditions when oxidation is slowly decreasing. The catalase stimulating substance decreases with decrease in oxidation, and therefore catalase falls off also. It would also decrease with decrease of rate of the metabolism which produces it.

After the consideration of these facts it is clear that the use of catalase as a direct measure of metabolic rate is valid only within certain limits. If catalase is formed as a response to some product of respiration, its curve will always follow, never precede, the respiration curve; that is, during comparatively slow changes in respiration rate, catalase will rise more slowly and fall more slowly than respiration, while in sudden changes it may actually decrease (or increase, doubtless) when respiration is doing the opposite. Catalase, therefore, could not be used as an indicator of respiration or metabolic rate immediately following a change in conditions that affects respiration suddenly. During slow changes

in respiration rate, however, it could at least afford a rough measure of oxidation rate, and when conditions are stable for respiration it could be used as a very convenient and reasonably accurate measure of this process.

Summary

1. Catalase decreases in early germination stages in six kinds of seeds tested. The initial decrease is followed by the rise generally reported for germination.

2. Catalase decreases slowly, almost to exhaustion in prolonged soaking of seeds in oxygen-free water.

3. There is a decrease of catalase in both the embryo and the endosperm of germinating wheat. The subsequent rise occurs not only in the embryo but also in the endosperm, although in a much less marked degree.

4. A simple respirometer is described, in which large quantities of material can be used, and consecutive readings taken at will on the same sample. By direct reading of the manometers both the oxygen and carbon dioxide can be determined without titration and with practically no calculation.

5. There is a large and immediate rise in respiration when seeds are put in germinating conditions. The early curve of respiration, therefore, diverges widely from the typical germination curve of catalase.

6. Catalase in ripening seeds decreases per unit both of wet and of dry weight. It increases per ovule as the seed grows, but after the ripening process begins, it decreases rapidly with decrease of water content.

7. The production of catalase is indirectly connected with oxidation, and LOEW's theory of the function of catalase is advanced to explain the facts, if the presence of some substance such as hydrogen peroxide, on which catalase acts, be formed to act as a stimulus for the production of the enzyme.

8. Catalase could be used as an indicator of metabolism only in cases where there is no rapid change in respiration.

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FLOWERS AND INSECTS. XXIII

CHARLES ROBERTSON

The flower groups are divided into (1) a social set, whose flowers are in heads, spikes, or close umbels, so that insects pass from one to another without taking wing or climbing; and (2) a non-social set. The latter contains 54.4 per cent of the flowers, and is divided into Ma, 121 long-tongued bee flowers, and 11 adapted to birds, hawk-moths, and butterflies; and Mi, 100 short-tongued bee flowers, and 6 adapted to flies. The social set includes Mas, 82 long-tongued bee flowers; Mis, 94 short-tongued bee flowers; and Pol, 23 visited by miscellaneous short-tongued insects.

The insect visits (13,971 pollinating and 850 non-pollinating) were first distributed under MÜLLER's (8) classes. These were then divided into non-social and social sets, and the visits were distributed again under them. Of 9 cases, maxima under Hb fell under non-social Hb in 6. In 27 other cases, however, maxima under B, AB, or A always fell under the social sets. As a criterion the social or non-social condition is of primary importance, while that of nectar concealment is secondary.

The flowers were also divided into a group of 235 plants observed before July, and one of 262 plants observed after June. Visits to these were first distributed under MÜLLER's classes. Before July there are maxima under B, AB (including Po), and A. After June there is a slight increase in Hb, including O and F, and a marked increase in B'. The visits were also distributed under the new classes. The early group shows maxima of the simplest flowers, Mi and white colors. These, along with Mis, decrease in the late group, which shows maxima of Ma and red (all dark colors), and a great increase in Mas. The specialization of the late group is marked by the increase of the social flowers from 38.7 to 54.1 per cent.

When B' as an element of the flora changes from 8.0 to 28.2 per cent, in 94.2 per cent of the cases the maxima of the insect groups change from the four other dominant classes to B'. The

maxima of the same insects, when distributed into early and late sets of the new categories, in 62.8 per cent of the cases fall under the same classes.

Importance of bees and method of investigation in anthecology

Of 2288 visitors and 13,971 visits, the Lepidoptera show 7.3 and 8.5 per cent, the flies 31.2 and 27.0, and the lower Hymenoptera 26.0 and 16.8. The bees, however, form 22.9 per cent of the species and make 43.3 per cent of the visits. In 22 cases of dominant orders, families, and genera, bees show a gain of visits over species, except in *Eupatorium*. Flies show a little gain for visits in only three cases, the lower Hymenoptera in six, and the Lepidoptera in four.

SPECIES AND INDIVIDUALS.—After a summary of insect visits to different sets of Compositae, MÜLLER (8) states:

This table proves clearly that in Compositae as the honey becomes more deeply placed the visits of more highly specialized bees increase, while, in spite of the exposed situation of the pollen, the visits of flies fall off. This is true of the number of species of visitors; but if it were possible to record the number of visits paid by each species, the preponderating influence of bees would be still more apparent.

This statement involves a criticism of basing conclusions on lists of species, a method which is quite unsatisfactory because it results in an underestimate of the importance of bees.

It is obvious that, if flowers are modified by their visitors, the number of individuals of each kind and the probability of repeated and diligent visits are very important. In the quotation MÜLLER implies that it is impossible to record the number of visits paid by each species. KNUTH says (5): "It is almost impossible to count all the individual visits that a conspicuous flower receives during a considerable period of observation, when the weather is fine." If one is making a list of the species which visit a given flower, one will look out for species not noted before, the rare and exceptional ones, and constantly exaggerate their importance. The longer the list, often the more erroneous the result. Generally it is easy to add new species, but a satisfactory census can be made in

one season. In the case of *Aster ericoides villosus*, the present list shows flies as 39.7 per cent of the visitors, and bees 30.8. In a census list the flies were 41.9 per cent of 124 species, but the bees 33. Of 1126 individuals, however, the flies were 33.5 per cent and the bees 49.2. When bees are 22.9 per cent of the total species and flies 31.2, in the case of flowers adapted to miscellaneous short-tongued insects, it is probable that, with continued watching, the percentage of fly species will increase. This may account for the fact that in the 1896 list of 116 species (11) the flies were 36.2 per cent, while in the present list of 292 species flies are 39.7.

In the case of *Rudbeckia subtomentosa*, the long-tongued bees showed 14.4 and the short-tongued 17.1 per cent of 76 species. Of 669 individuals, the former were 6.5 per cent and the latter 40.5. In the case of 87 species, taken on *Symphoricarpos orbiculatus*, bees were 35.6 per cent and wasps 34.4; while of 1065 individuals, bees were 76.3 per cent and wasps 9.8. Of 45 species, taken on *Lippia lanceolata*, bees were 33.3 per cent, flies 42.2, and wasps 8.8; while of 288 individuals, bees were 44.7, flies 29.1, and wasps 3.4 per cent. In 60 cases in which insects were taken as they came, bees gained in percentage of individuals over that of species in 53. Of 405 species observed on 41 plants, bees were 31.6 per cent; but of 7391 individuals, bees were 57.4 per cent. Insects of the other groups declined.

Forty-three plants with *Diptera maxima* showed for species and visits of bees 21.1 and 25.5; of flies 36.3 and 42.9. Twelve of these showed for visits and individuals of bees 32.9 and 49.5; of flies 42.9 and 35.3, a gain for bees and a loss for flies. Twenty-six plants with lower Hymenoptera maxima showed for species and visits of bees 20.2 and 21.4; of lower Hymenoptera 35.6 and 40.4. Seven of these showed for visits and individuals of bees 28.3 and 65.7; of lower Hymenoptera 37.2 and 14.6, a gain for bees and loss for lower Hymenoptera.

THE COMMONEST VISITOR.—In lists of species some are marked "abundant" or "frequent." What these terms amount to is shown when the individuals are counted. A bee, which makes only one in a list of species, often shows a remarkable importance as a pollinator. Sometimes this may be owing to the fact that

the flower blooms when the bee is most active. At Inverness, Florida, 1000 individuals taken on *Laurocerasus caroliniana* showed 527 of *Opandrena scutellaris*. Another 1000 on *Fagara clavaherculis* showed 256 individuals of *Colletes thoracicus*, and only three of another *Colletes*. *Odontalictus capitosus* formed 65.3 per cent of 300 individuals taken on *Berlandiera subacaulis*, and 17.8 per cent of 599 individuals on *Thysanella fimbriata*. The first 100 individuals on *Claytonia virginica* showed 39 specimens of the oligolectic *Ptilandrena erigeniae*. In 85.5 per cent of local flowers, on which the individuals were taken, the commonest visitor was a bee which showed 3.5 per cent of the visits and 20.7 per cent of the individuals.

ORDINARY AND CENSUS VISITS.—When the visitors are captured without discrimination instead of being observed to ascertain the number of species, bees usually show higher percentages of species and visits. The visits to 29 plants showed 32.1 per cent of bees, but the same plants, when the individuals were taken as they came, showed 38.8 per cent of bees.

To make a correct statement regarding a flower visited by miscellaneous insects, a census of species and individuals taken without discrimination ought to be made about every other day throughout the blooming season.

APOCYNACEAE AND ASCLEPIADACEAE.—In 11 species bees showed 21.6 per cent of 273 visits without pollinia, and 32.1 per cent of 308 visits with pollinia.

DICLINOUS FLOWERS.—In 8 species of diclinous plants bees made 37.8 per cent of 420 visits to staminate or pistillate flowers only, and 63.4 per cent of 194 visits to both kinds.

Imperfect adaptation of flowers to insects

Flowers may be regarded as showing imperfect adaptation whenever they fail to exclude visitors which are unable to reach the nectar in the normal way, or which obtain the nectar or pollen without effecting pollination. More than 38.2 per cent of the flowers considered here show such imperfect adaptation. When the indigenous flora and the insects which depend upon the flowers are being rapidly exterminated, the relations are inevitably more

or less out of joint, and cases of imperfect adjustment become more frequent. As regards the existence of flowers which have no unbidden guests, however, or of floral structures which always effectually exclude such guests, it is hardly an exaggeration to state that there are none.

Flowers with exposed nectar admit the smallest insects. Such flowers are usually small, but some of their visitors are so minute that they can obtain the nectar without pollination. The larger the flowers, the greater the possible number of unbidden guests. On *Melanthium virginicum* fifteen visitors were observed sucking without carrying pollen. As many as twenty Chironomidae were observed on a single nectary. *Liparis liliifolia* and *Caulophyllum thalictroides* have similar useless visitors.

Many flowers whose nectar is partly concealed or rather deep seated admit insects too small to touch the anthers and stigmas, such as *Polygonatum commutatum* and *Geranium maculatum*. In some of these there appears to be a well marked lack of correlation between the size of the flower and the depth of the nectar.

In *Iris* (15) butterflies insert their proboscides between the sepals and petaloid styles and steal the nectar. In *Lobelia* (14) the stamen tube is not completely closed at the base. In *L. cardinalis*, *Bombus americanorum*, which cannot reach the nectar in the legitimate way, obtains it by inserting its proboscis through the slits.

In the case of *Cypripedium pubescens* two species of Ceratinidae were seen entering the labellum and making their exit in the normal way without extracting the pollen. These bees were evidently too small.

The bearded violets are adapted to small bees which reverse and insert their proboscides under the anthers and stigma. Some bumblebees, long-tongued flies, and Lepidoptera fail to reverse and so extract the nectar without effecting pollination.

Flowers with concealed pollen may be pollinated by Lepidoptera when the anthers are inclosed in slender tubes, as in *Phlox*, but in other cases they usually fail to exclude such insects, although they are not pollinated by them.

Personate, papilionaceous, closed flowers, like *Gentiana Andrewsii*, *Collinsia verna*, and *Dicentra cucullaria*, and flowers with the

throat closed by tufts of hair, like *Seymeria macrophylla*, or with the stamens protected by petals, as in *Delphinium tricornis*, usually exclude all unbidden guests, except Lepidoptera and siphonate Diptera. *Linaria vulgaris* is adapted to bumblebees, which are heavy enough to pull down the palate easily, less so to smaller bees, which have to squeeze in sideways, and not at all to butterflies, which steal the nectar.

Apocynum fastens its pollinia to the maxillary and labial palps of bees and wasps, but can neither utilize nor exclude flies and Lepidoptera.

Asclepias admits many insects which get the nectar without extracting the pollinia, and others which have difficulty in breaking the retinacula or freeing their claws from the slits, and so often are entrapped and killed. Eleven species of Apocynaceae and Asclepiadaceae fail to utilize 46.9 per cent of the visits made to them.

As a rule visitors of one form of diclinous flowers are admitted here as legitimate on the assumption that they probably occur on both forms, but in elucidating floral mechanisms, only those visitors which were found on both forms should be admitted. The Syrphidae and Coleoptera show a marked preference for the staminate flowers. In 614 visits to eight species of diclinous flowers, 68.4 per cent were to one form.

In the case of the staminate flowers of *Diospyros virginiana*, the Syrphidae made 18.7 per cent of the visits, avoiding the pistillate. Short-tongued bees made 62.7 per cent of the visits to staminate flowers, and 33.3 of the visits to both forms, but the two forms look as if they belonged to distinct species. The staminate are smaller and have less deep seated nectar.

The staminate flowers of *Salix humilis* have been observed to attract hive-bees in such numbers as fairly to monopolize them, while the pistillate flowers were visited by an entirely different set of insects.

Dichogamous flowers which are discharging pollen are often more attractive to insects than those in the other stage. *Impatiens biflora* in the first stage induces pollen visits of some long-tongued bees which avoid the flowers with receptive stigmas. *Campanula americana* attracts *Oligotropus campanulae* before the stigma opens, but not after. *Lobelia siphilitica* invites the visits of little bees,

which collect the pollen which is pushed out of the anther tube before the stigma appears. *Monarda bradburiana* admits small bees to which the nectar is inaccessible, but they collect pollen directly from the anthers, avoiding the old flowers. Thirty-five species of flowers have been observed to attract bees which collect pollen in the first stage but avoid them in the second.

Personate, papilionaceous, and other closed flowers usually exclude pollen-hunting insects which cannot reach the nectar, but *Dicentra cucullaria*, *Delphinium tricornis*, and *Trifolium pratense* sometimes fail to do so in spite of hindering structures.

As a rule flowers with exposed pollen and deep seated nectar, like *Abutilon Theophrasti* and *Mertensia virginica*, are visited for pollen by small insects, usually Halictidae and Syrphidae. On the bird flower, *Tecoma radicans*, and the hawk-moth flower, *Datura tatula*, bees were observed collecting pollen.

Large pollen flowers, such as *Tradescantia*, *Rosa*, and *Cassia*, which are adapted to large bees, are commonly visited by small bees, Syrphidae, and beetles which do not often effect pollination.

Ordinary flowers with exposed pollen probably attract more Syrphidae than any other useless visitors. These flies are light and rest so superficially on the flowers that they can eat the pollen without carrying it.

Many flowers fail to exclude beetles, which eat the pollen or gnaw and often completely destroy various flower parts.

Some flowers are visited by *Megachile brevis*, when she cuts circular pieces out of the petals to line her nests.

In the Alps, flowers whose nectar it could not obtain in the normal way are often perforated by *Bombus maurus* (MÜLLER 9). In the Tyrol, SCHULZ (12) found 125 species of flowers perforated by *Bombus terrestris*. This may be a result of the competition with the domestic hive-bee on flowers to which this bumblebee would normally confine its visits. In observing the visits of bumblebees to 278 insect flowers, none was ever seen perforating, and none obtaining nectar in an altogether irregular way except on *Lobelia cardinalis*. No flower was seen to be perforated except by *Leionotus foraminatus* and *L. dorsalis*.

Flower buds often contain nectar before they open. I have seen the rubythroat thrust its bill into buds of *Lonicera sempervirens*,

and *Bombus consimilis* force its proboscis into buds of *Triosteum perfoliatum*. This may help to account for cases like *Gentiana Andrewsii*, whose flowers never open.

Homogamous flowers, which are so small that insects cannot obtain the nectar or collect the pollen without effecting pollination, seem to be best adapted to utilize all of the visitors which they attract.

The useless visits to flowers were made by Lepidoptera, Halictidae, Syrphidae, and Coleoptera, respectively 20.5, 19.3, 17.0, and 14.4 per cent of the total, together 71.2 per cent. These useless visits, however, are only 5.7 per cent of the total visits observed. Of these visits, 41.1 per cent are to Ma, 31.3 to Mas, and 46.3 to red, that is, to the flowers with the deepest seated nectar.

Characteristics of insect flowers

NECTAR.—The existence of "nectar covers" is not obvious. There are structures which have that effect, but they exist also in pendulous flowers where they would not be needed. Nectar, together with pollen and other parts of the flower, is protected by the closing of the flower in rainy weather. The structures called "nectar covers" should probably be regarded as serving to exclude the less efficient pollinators.

Some flowers, as *Orchis*, are said to have no free nectar in their spurs. Insects are supposed to obtain the nectar by boring into the juicy cellular tissue. In *Habenaria leucophaea* and *Orchis spectabilis*, nectar rises so that the height can be told from the outside. This hardly supports the idea that the nectar is in the cellular tissue.

That glandular hairs in pollen flowers (KERNER 4) supply means of moistening the pollen is altogether doubtful. Such hairs exist in *Steironema*, which *Macropis steironematis* visits for pollen, but this bee can easily moisten the pollen with honey obtained from the several flowers which it visits for nectar.

POLLEN.—Pollen often influences the visits of insects to flowers. Female bees, with their plumose, hairy coats covered with it, are the most certain of all insects in pollinating, so effective that many flowers have lost their nectaries and usually are visited exclusively by female bees. After the bees, the Syrphidae and beetles are the

most frequent visitors for pollen, which they can eat without much certainty of effecting pollination.

KERNER (4) has given seventeen arrangements for protecting pollen. Pollen is certainly sheltered by a pendulous position of the flower, but it does not follow that any plants were selected because their pollen was protected by that position. The closing and nodding of flowers at night and in bad weather may be regarded as having some connection with natural selection to protect the floral organs, but not the pollen exclusively. The spathe of *Arisaema triphyllum*, mentioned by KERNER as protecting pollen, also protects the flowers which have pistils, but no pollen. "In *Podophyllum peltatum* the pollen is sheltered by the bell-shaped flower, but in addition to this, the peltate foliage leaves are also spread out over the flowers and act as umbrellas."

The anther closing, or remaining closed in moist weather, and arrangements in which the pollen is retained until dislodged by the legitimate visitors, may be regarded as pollen protective: the triggered anthers of *Gerardia grandiflora*, the milked anthers of *Cassia*, the bellows-like anthers of *Rhexia*, the anther cones of *Viola* which shed when the style is touched, the keel of Papilionaceae, galea of Labiatae, closed flowers, etc. Pollinia are usually retained until dragged out by insects, but the protective advantage is rather incidental.

In wind flowers the pollen is light and dry, and easily carried by the wind. In some insect flowers the pollen is shed upon the visitors and is dry and powdery; commonly it is rough and more or less viscid. Sometimes the grains are connected by threads, as in *Oenothera*, or the contents of an anther cell may be united into a pollinium.

In *Cassia chamaecrista* some anthers serve to supply pollen to the guests, and others to furnish it for cross pollination.

Ordinary flowers dust the insect with pollen quite indefinitely, but mainly on the lower side. Some gonous zygomorphous flowers dust the insect on the back, some on the ventral surface, and some on the side. In Florida an *Astragalus* was observed whose keel was always turned to one side, so that it dusted the bees only on the under side of the left cheek. *Orchis spectabilis* fastens its pollinia

to the bare clypeus of female bumblebees, *O. pyramidalis* (1) to the upper side of the proboscides of Lepidoptera, *Habenaria leucophaea* to the tongues, and *H. orbiculata* (3) to the eyes of Sphingidae, *Spiranthes gracilis* to the maxillary galea of bees, *Calopogon parviflorus* to the first dorsal segment of the abdomen. *Asclepias Sullivantii* fastens its pollinia to the claws, *A. verticillata* to the tarsal hairs, *Acerates floridana* to the ventral hairs, *Gonolobus laevis* to the tongues, and *Apocynum* to the palpi.

In addition to nectar flowers and pollen flowers may be distinguished the fruit flowers of *Yucca* and the gall flowers of *Ficus*. In these the fruit is partly injured by the pollinator.

COLOR.—Usually the petals, sometimes the sepals, or both, serve as advertising structures, and are variously colored.

Nectar guides, colored marks contrasting with the ground color of the flower, and indicating the situation of the nectar, were designated by SPRENGEL (13). They facilitate effective insect visits. Usually flowers with these guiding marks are zygomorphous, or otherwise complicated, or with deep-seated nectar. They are common in Papilionaceae, Lamiales, and Personales. On *Lantana odorata*, butterflies insert their proboscides into the flowers with guides, but avoid the old flowers, in which the guides are obliterated. It seems evident that the insect can distinguish these flowers only by the color.

Pollen flowers also have guiding marks called path finders. They seem to occur only in zygomorphous flowers, *Desmodium*, *Cassia*, and *Calopogon*. In *Desmodium* bees insert their heads under the specially marked vexillum when they force down the wings. In *Cassia* the path finders mark the location of the short stamens. Colored marks are present in *C. chamaecrista*. These are absent in *C. marilandica*, but three stamens are reduced to broad, dark scales to take their places.

Evidently the importance of the colors of flowers has been exaggerated. They should be regarded as advertisements rather than attractions. Insects are guided by the colors in their search for floral food, but they do not visit flowers to look at their colors. To humanize insects, it may be stated that they visit flowers for the following purposes: (1) for nectar; (2) in search of nectar which

the flowers do not contain (*Lycaena*, *Coelioxys*, *Anthrax* on *Desmodium*); visits to sham-nectar flowers; visits of various insects to flowers whose nectar they cannot reach; (3) for pollen, with which they provision their nests (bees); which they eat (*Syrphidae*, beetles, etc.); (4) for pollen which cannot be collected; female bees on sham-pollen flowers (*Calopogon*); (5) to cut holes in flowers whose nectar they cannot reach normally (*Leionotus foraminatus*); (6) to get nectar from holes made by *Leionotus*; (7) to gnaw floral organs (beetles); (8) in search of anthophilous insects (*Vespa*, *Polistes*, *Protothyreopus*, *Ochthera*); (9) for flower spiders (*Sceliphron cementarius*); (10) to lie in wait for anthophilous insects (*Phymata*); (11) in search of the females; male bees often fly around among the flowers, only approaching near enough to discover the presence of the females; (12) to deposit their eggs in the ovaries (*Pronuba*, *Blastophaga*); (13) for shelter; flies in *Arisaema*; bees in various flowers; (14) to deposit eggs so that their larvae may become attached to anthophilous hosts (*Rhipiphoridae*); (15) to cut petals to line their nests (*Megachile brevis*); (16) merely as resting places.

That insects can find nectar without any guides is shown by the great variety of species (22) that may be found on sugar trees from which the sap is exuding, on leaves covered with honey dew, or on extranuptial nectaries, 16 species on *Tecoma radicans*, 66 on *Cassia chamaecrista*, and 43 on *Strophostyles helvola*.¹

The readiness with which insects find nectar is also shown in the case of *Monarda fistulosa*, in which nine species of bees and one wasp, which could not reach the nectar in the normal way and which consequently could hardly be regarded as attracted by the colors of the flowers, were observed sucking nectar through holes made by *Leionotus* (10).

Anemophilous flowers which have no conspicuous colors are visited by bees for pollen.

Nevertheless, that the colors of flowers guide insects to them is shown by the frequent visits of insects to flowers which do not contain anything they can get; visits of butterflies, inquiline bees,

¹ The bees taken at sugar maples were *Apis mellifera* and *Trachandrena rugosa*; those on the extranuptial nectaries were usually *Halictidae*. Of these insects, eleven species of lower Hymenoptera and six species of flies were never taken on any flower.

and siphonate flies to pollen flowers; of bees to sham-pollen flowers; of flies to sham nectar flowers. DELPINO (2) observed a bee working on *Anemone nemorosa* confused by the presence of *Bellis perennis*, which it often approached before recognizing its error, and I have often noticed bees confused by the presence of similarly colored flowers which were mixed among those on which they were working. On several occasions *Bombus americanorum* was observed approaching pappus heads of *Taraxacum officinale*, evidently mistaking them for flowers. LUBBOCK (6) has shown that hive-bees can distinguish colors and use the colors to enable them to return to the same place where they have found honey.

Another fact bearing on this subject is connected with the colors of spiders. The flower spiders, Thomisidae, are yellow or white, corresponding with the colors of the flowers on which they lie in wait. They have no other means of concealment, but a black jumping spider, observed on a head of *Helianthus*, formed a hiding place by tying several rays together.

A typical case showing the relations of insects to flower colors is that of the mud-dauber, *Sceliphron cementarius*. It visits for nectar 16 white, 6 yellow, and 3 red flowers, evidently aided by the conspicuous colors, but it provisions its nest with flower spiders in spite of their cryptic colors. Of the organisms from which insects get their food, flowers are almost the only ones which gain any advantage from their attentions and which facilitate their search.

COLOR PREFERENCE OF INSECTS.—The only direct observation on color preference was in the case of a hawk-moth, *Celerio lineata*, visiting a bed of petunias in which white and purple flowers were about equally abundant. It distinctly avoided the purple flowers and visited the white ones.

There are indications that insects have had color preferences which have influenced the colors of their favorite flowers, but their visits to certain colors are always complicated with other conditions. We often read about reactions of insects as if they were quite simple. To prove this simplicity we have to go back to the first generation, or show that natural selection, heredity, and experience have nothing to do with the cases.

As we pass from red through yellow to white, the visits and the average number of visits increase. The visits of long-tongued bees, of bees in general, and of Lepidoptera decline; the visits of flies, lower Hymenoptera, and short-tongued bees increase.

COLOR PREFERENCE OF BEES.—MÜLLER says: "On the whole we find red, violet, and blue colors appearing for the first time in flowers whose honey is quite concealed and which are visited by more or less long-tongued insects (bees, etc.)."

LUTZ (7) states that bees are largely given to visiting blue, purple, or violet flowers, although not totally ignoring yellow or red ones. Bees are heterotropic. They have become diversified in correlation with the diversification of the entomophilous flora. Only 42.7 per cent of indigenous entomophilous flowers bloom at the same time, and only 47.2 per cent of the bees fly simultaneously. It is not probable that 296 species of local bees are so free from competition among themselves that they do not have to trouble themselves about flowers, except those whose colors suit their fancy. Bees visit nectar-bearing flowers of all kinds, except those adapted to birds, hawk-moths, and flies, which are so constructed that they are practically excluded. Even on these, bees are apt to force their attentions, as in *Lobelia cardinalis*, *Tecoma radicans*, and *Datura tatula*. There are only three local flowers on which I am very sure bees never occur: *Habenaria leucophaea*, *Aristolochia tomentosa*, and *Trillium sessile*. If we include non-pollinating visits, the percentage of flowers visited by bees is 96.2.

In the case of the 437 local flowers the colors are red 30.2, white 39.1, yellow 30.6. The 417 flowers visited by bees are red 28.7, white 40.0, yellow 31.1. The 6063 bee visits are red 23.1, white 43.2, yellow 33.5. The flowers visited and the visits made are not very different from what might be expected without regard to color.

Of local bees only 70 (23.6 per cent) are largely given to visiting red flowers. Of their visits, 45.9 per cent are to red; of the visits of 130 other bees, 55.9 per cent are to white; while of the visits of the remaining 96 species, 52.9 per cent are to yellow.

There is a little more reason for KNUTH'S (5) statement that long-tongued bees prefer red, blue, and violet colors. In the case of 146 local species, however, only 60 (41.0 per cent) prefer red, 46.0 per cent of their visits being to that color. Forty-three show

a maximum of 50.3 under white, and 43 show a maximum of 51.4 under yellow.

The long-tongued bees show for flowers visited red 31.6, white 37.2, yellow 31.0. The visits show: red 34.0, white 34.5, yellow 31.4; so that their visits are about what might be expected without color preference.

There are not many flowers differing in colors but alike in other respects. In Ma I include 121 long-tongued bee flowers whose colors are red 56.1, white 21.4, yellow 22.3. Long-tongued bee visits (688) to these show red 52.6, white 24.1, yellow 23.2; about what might be expected without color preference. The percentages of long-tongued bee visits to the three colored sets of flowers of this class are to red 59.7, white 78.6, and yellow 73.0. Before July 29.6 per cent of the flowers of Ma are white and 20.3 yellow, after June 13.7 are white and 26.0 yellow. Visits of long-tongued bees to this class showed before July, 30.5 per cent under white and 16.0 under yellow, after June, 13.1 under white and 31.5 under yellow, so that when conditions are equal, long-tongued bees will make about the same percentage of visits to flowers of one color as to those of another. If the flowers of class Ma were separated into those with ultra violet colors and those without them, the visits to each set would correspond fairly well with the percentages of flowers. Of 30 species of bees showing maxima, or principal preferences, under Ma, 20 showed similar maxima, or preferences, under red.

In the case of the rubythroat, 82.7 per cent of the visits are to general Ma, which shows 57.5 per cent of red flowers. Of its visits, 55.1 per cent are to red, which is about what might be expected without color preference. This bird, however, seems to prefer bright red, or scarlet, all of our bird flowers being of that color.

Flowers referred to Mas are red 43.9, white 10.9, yellow 45.1. Visits of long-tongued bees (1185) show red 45.5, white 9.4, yellow 43.9. Of 100 bees showing maxima, or principal preferences, under Mas, 44 showed similar maxima, or preferences, under red, 9 under white, and 47 under yellow. Long-tongued bee visits to red, white, or yellow Mas differ less than 1 per cent.

In 87 *Asterales* the radiate species were classed according to the color of the rays. In general, red shows the longest tubes

and white the shortest, while yellow shows tubes of about average length. Accordingly, red shows a loss and white a greater gain than yellow. The colors are red 21.8 per cent, white 31.0, yellow 47.1; while 3939 visits of insects in general show red 13.6, white 37.4, and yellow 48.8. The visits are about what should be expected according to the depth of the tubes and without regard to the colors.

Of the visits of Bombinae, 41.1 per cent are to red flowers and 30.1 to white, while of the visits of the Halictidae 50.0 are to white flowers and 14.4 to red. There seems to be no evidence that the color is the determining condition. The inference of color preference rests on the fact that the visits of the former show a higher percentage to red flowers, and of the latter a higher percentage to white ones. If we assume that in the first place the Bombinae preferred red and the Halictidae white, the color factor must have ceased to become dominant after the flowers became modified to suit the one, and exclude, or fail to satisfy, the other. The fact that the Halictidae make 86 visits to red flowers, although they cannot obtain the nectar in the legitimate way, establishes the presumption that they would be more frequent, if they could obtain the nectar. Also, the bumblebees would visit more white flowers if these flowers afforded a more abundant supply of nectar.

Oligolectic bees might be expected to show color preferences according to the colors of their special flowers. On their visits to unrelated flowers, however, the short-tongued oligoleges of red and yellow flowers prefer white. In a similar way the nectar visits to unrelated flowers of long-tongued oligoleges show preference for red flowers without regard to the colors of the flowers which they visit exclusively for pollen. The long-tongued oligoleges in 127 pollen visits show red 23.6, white 14.1, yellow 62.2; in 142 nectar visits to unrelated flowers they show red 59.1, white 27.4, yellow 13.5. The short-tongued oligoleges in 187 pollen visits show red 13.3, white 19.2, yellow 67.3; in 109 nectar visits to unrelated flowers they show red 7.3, white 70.6, yellow 22.0.

In the case of the 235 plants observed before July, the colors are red 26.3, white 44.6, yellow 28.9; while of the 262 plants observed after June the colors are red 34.3, white 33.5, yellow 32.0.

Of 19 groups of bees whose visits were distributed under these two sets of flowers, 8 show a change of color in their maxima. Before July, 14 show maxima under white and 5 under red. After June, 8 show maxima under white, 6 under red, and 5 under yellow. The changes are determined by the composition of the flora, and have little to do with color preference. When the maxima change from other classes to B', 47.1 per cent of whose flowers are yellow, there is a probability that some will fall under yellow.

According to LUBBOCK (6), the hive-bee prefers blue. When about three-fourths of the flowers whose nectar it can reach are white or yellow, how is a bee which flies all season and gathers so much honey going to limit itself to blue flowers? Of 196 local visits, 24.4 per cent are to red, 43.3 to white, and 32.1 to yellow.

It is an interesting fact that when bees become restricted to certain flowers, the preference is determined by the natural relationship of the flowers, and not by their colors. Of 83 local oligolectic bees, in spite of the fact that the restriction would be expected to limit the flowers to one color, 31 get their pollen from flowers of more than one color.

The first thing that the anthecologist learns is that the most important condition of insect visits is the presence of a supply of nectar and pollen, and that the most favorable time for observing insect visits is early in the morning, or soon after the flowers open. Bees clean up the pollen and nectar, and leave the flowers, which still remain as conspicuous as ever. In many flowers, like *Tradescantia* and *Desmodium*, the large bees are followed by small bees and Syrphidae in search of stray pollen.

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CELLULAR STRUCTURE OF TENDRILS

HENRIETTA LISK

(WITH FIVE FIGURES)

Although the physiology of tendrils has been exhaustively studied, comparatively little work of an anatomical nature has been done. In reviewing the literature included in this paper, all references are to the structure of tendrils in general, unless specific examples are given.

PENHALLOW (6), in investigating the movements of *Cucurbita maxima* and *C. Pepo* tendrils, considered at some length their histological structure. In cross-sections of squash tendrils he found beneath the epidermis a somewhat thick layer of collenchyma, its continuity broken at three points by parenchyma tissue. The three parenchyma regions were found upon the upper side and the right and left. The cells of these regions were large, rounded, and somewhat thin-walled. They contained protoplasm and considerable chlorophyll. Within these areas were intercellular spaces with corresponding stomata in the epidermis. PENHALLOW found that in these parenchyma regions the greatest growth occurred without regular order, but in turn. He gave to this tissue the name vibrogen, signifying that the region of circumnutation was to be found there.

Within the layer of collenchyma were found three or four rows of rather large and rounded parenchyma cells. These cells were filled with "protoplasm, chlorophyll, and other granular matter." The amount of chlorophyll was much less than in the vibrogen region with which this tissue was connected. Beneath the parenchyma were found wood cells. In the early period of growth of the tendril, the wood tissue formed a crescent along the lower side. Later, breaks were found in the tissue corresponding to the position of the vibrogen regions. Finally these breaks were found to close up, and the tissue became a ring of wood. With age the wood cells developed thick walls. Next in the remaining tissue were found the fibrovascular bundles, seven in number. The central pith

was often seen to be shrunken, leaving the basal portion of the tendril hollow.

WORGITZKY (8), as a result of his investigations, came to the conclusion that the structure of tendrils stood in close relation to their function. As a demonstration of the fact that the main functions of tendrils were mechanical, he pointed out that a tendril which did not reach a support formed a spiral and died. As mechanical demands differed before and after the tendril surrounded a support, changes in structure were necessary. Differences in structure were found in the portion surrounding a support and the portion forming a spiral, for stiffness was necessary in the one, and elasticity in the other. Both portions were alike, however, in the unilateral development of the side which became attached, thus inducing dorsiventrality. Dorsiventrality, WORGITZKY found, was brought about in two ways: in the anlage, the anatomical structure was radial, and dorsiventrality appeared only when one-sided demands were made upon the curved portion of the tendril; or the arrangement of tissues in the very youngest stages was dorsiventral. The first was the case in tendrils showing secondary thickening, and here the dorsiventrality was brought about through the activity of the cambium. Where dorsiventrality was present in the very beginning, as in the majority of cases, WORGITZKY considered that this was due to the development of the tendril from a dorsiventral organ, as the blade of a leaf. He further concluded that the manner in which dorsiventrality was brought about differed in different tendrils, and in different regions of the same tendril. He found it true almost without exception that in the spirally twisted portion the mechanical elements lay on the concave side. The tissues of the concave side developed a cushion of tissue, which served to protect the tendril from the support, and at the same time brought about a closer union between tendril and support. The cells of the concave side were often stretched in a radial direction. This was conspicuous in the cortical parenchyma and collenchyma of the Cucurbitaceae.

Although the Cucurbitaceae were among these tendrils showing dorsiventrality in the earliest stages, they often showed a radial structure in the basal region. In these tendrils the dorsiventrality

was brought about primarily through the strands of mestome. The largest bundle lay on the concave side, and on either side, in a half moon shape, the smaller bundles were arranged. The bundles were almost always of an uneven number in the upper portion of the tendril (usually 5-7), and an even number in the base. The distribution of bast and collenchyma was found to follow that of the bundles. The changes which occurred after coiling around a support, and which made dorsiventrality more conspicuous, took place outside the bundles, and especially in the portion lying against the support. The indentation, which was found on the side becoming convex, flattened out as the tip was neared.

In the Cucurbitaceae, a peculiar forking of the bicollateral bundle, without cambium, was noticeable. The newly formed bundles finally weakened and disappeared, or united with others. The rule seemed to be for five bundles in the curved portion of the tendril, no matter how many at base, middle, or tip. The bast was found to form a complete ring in the basal portion of the tendril, or to have an opening on that side which in the upper region was to become convex. The complete ring was usually found in those tendrils which did not develop secondary thickening. In the Cucurbitaceae bast was often arranged in strands. In *Cucumis sativa* it was found with an opening of the ring on the convex side. Often transitional stages between true bast and true parenchyma (pith) were observed.

WORGITZKY found collenchyma to be typical for most young tendrils; either present in a closed ring, or in parallel bands stretched tangentially rather than radially. From the fact that collenchyma was often in isolated strands in the basal portion, and in a continuous ring in the middle and tip portion, he concluded that the arrangement of collenchyma bore a relation to the mechanical function of the tendril, for a closed ring could not only withstand bending but torsion also. Collenchyma arranged in isolated bands was found only in those tendrils having angles, and only in these angles. Tendrils having an indentation on the side becoming convex had collenchyma strands on each side of the indentation. The number of strands was found to coincide with the number of large bundles or groups of bundles. While in the young tendril, the collenchyma

had great mechanical significance; it lost its importance after the tendril surrounded a support. The formation of the closed ring of xylem had now begun, and these cells took up the mechanical function of the tendril. In all cases where collenchyma was present, the cortical parenchyma had less chlorophyll and fewer intercellular spaces. The cells were long and prismatic, and arranged in rows. The nearer the tip, the more noticeable was the rarity of chlorophyll in the cortical parenchyma. He also noted that chlorophyll was found in quantity only when the parenchyma extended to the epidermis. Where epidermis and cortical parenchyma came in contact was often the only place where stomata were found in the epidermis. The pith parenchyma consisted of large cells with large intercellular spaces. In the region where the tendril attached itself to a support, the pith became thick walled.

MÜLLER (5), in his researches on the tendrils of Cucurbitaceae considered the relation between the behavior of tendrils and their anatomy. He concluded that curvature accompanied dorsiventrality. In support of this theory he noted the following points. The basal, insensitive portion of the tendril had central structure; the upper sensitive portion was bilateral. In the first the sclerenchyma was closed; in the second it was open. In the first the bundles were distributed equally on all sides; in the second they lay more and more on the concave. So far as the tendril had central structure it could not bend, but curved where it was bilateral. Sensitiveness increased toward the tip, as did the bilateral structure. The nearer the tip was approached, the more flattened was the structure, and the more narrow the sclerenchyma. Thinness of the tendril, thinness of cell walls, and transparency of cell walls were all factors which furthered sensitiveness.

In studying the arrangement of tissues in the tendril, MÜLLER pointed out that those structures easy of change were on the convex side, while the more resistant structures were on the concave. The sclerenchyma was on the concave side. The larger vascular bundle lay on the concave side, and the other bundles lay more and more on this side as they approached the tip. From base to tip of the tendril there was a broad strip of collenchyma on the concave side, while on the convex side there were found large thin-walled

parenchyma cells. The upper convex side, as most easily changed, could take up liquids, and become stretched. MÜLLER accordingly believed that his anatomical results showed that curvature was due to elongation of the convex surface, and not to contraction of the concave. As curvature followed only when stimulus was applied to the lower side, he suggested that the collenchyma must first take up the transmission of stimulus, then pass it to the parenchyma, either through the sclerenchyma or to the right and left of it. As the parenchyma was most subject to pressure it transmitted the stimulus.

MÜLLER found that true sclerenchyma was present in the bud in the tendrils of *Cucurbita* and *Bryonia*. In *Cyclanthera* and *Sicyos*, sclerenchyma was not observed until the tendrils became sensitive. So long as the tendrils were sensitive, the sclerenchyma was not lignified. When lignification set in, it began in the center of the concave side, and extended to the right and left. MÜLLER often found the lower sclerenchyma entirely lignified, while the upper showed no traces of lignification. He also noted that the xylem became lignified before the sclerenchyma, and that the sclerenchyma of the tendril became lignified before that of the stem adjoining.

In taking up the anatomical changes in that portion of the tendril which has surrounded a support, MÜLLER found that this region became swollen, hard, and brittle. The swelling was brought about by an enlargement of the cells of the epidermis, collenchyma, and the parenchyma lying between the sclerenchyma and the periphery. There was no cell division, but simply an increase in the size of the cells.

In comparing the hardening of the portion of the tendril which surrounds a support and that portion which forms a spiral, MÜLLER found that in the former the entire central parenchyma became lignified. The sclerenchyma formed a complete ring, the upper side of which was often found to be the thicker. This ring was the result of gradual sclerenchymatous thickening of parenchyma cells. On the lower side and on the right and left, the new sclerenchyma ring was bounded by the old sclerenchyma. On the upper side, however, the ring often lay directly beneath the epidermis. All tissues within

this ring were found to become lignified, with the exception of the phloem. The epidermis, collenchyma, and parenchyma, outside of the ring, usually did not become lignified. In the portion of the tendril which formed a spiral, only sclerenchyma and xylem became lignified, consequently the tendril remained elastic at this point.

Among the Cucurbitaceae which MÜLLER took up specifically was *Cyclanthera pedata*. Of these tendrils, he studied both branched and simple. In the branched tendrils he noted the following characteristics: The main axis was usually six-sided, but not entirely central in structure. Two of the angles were much smaller than the other four. The collenchyma lay regularly on the outer edges of the angles. The layer of chlorophyll cells contained much of this substance. The sclerenchyma described six curves, and in each arc lay a bundle, the two smallest bundles lying in the smallest arcs. The four largest bundles were arranged in the form of a right angled cross. Between the six bundles in the basal portion of the tendril were seen six smaller bundles of few cells. These disappeared toward the tip.

The branch of the tendrils studied was found to have five angles, the lowest of which was the largest. Collenchyma lay in the angles. The sclerenchyma was open on the upper side, and described five arcs corresponding to the angles. Five bundles lay in the angles, and sometimes a sixth appeared in the opening of the sclerenchyma. As the tip was approached the largest angle became more and more flattened, until the circumference of the cross-section was almost square. The collenchyma remained until very near the tip. Openings appeared in the sides of the sclerenchyma ring, and the original upper opening became larger. The arcs finally disappeared toward the tip.

A cross-section of the base of a simple tendril showed a four-angled structure with collenchyma in the corners. Next lay the parenchyma cells containing chlorophyll, and a closed ring of sclerenchyma corresponding in shape to the circumference of the tendril. The four bundles lying in the angles were usually nearly the same size, with sometimes the lower the largest, and the upper the smallest. In the sensitive portion of the tendril, the upper

bundle broadened and became heart-shaped. From this two bundles were formed. As these bundles separated, the angle in which they lay became broader. The collenchyma split in two parts, and an indentation was formed between these parts, which became deeper as the circumference of the tendril became five-angled instead of four. The sclerenchyma also split between the two newly formed angles. The cross-section of the tip of a simple tendril was formed to resemble that of the base of a tendril branch.

MACDOUGAL (3), working with the tendrils of *Passiflora caerulea*, had for his purpose the determining of "the factors in the movements of the tendrils of the *Passiflorae*, more particularly the movements by which a tendril responds to a stimulus, resúmes its original position, or on continuance of the irritation coils permanently." He also investigated the subsequent changes in the tendrils while coiled and serving as a support for the adjacent parts of the plant. To determine the conditions prevailing in the tendril during the sensitive period, MACDOUGAL studied the development through all stages of growth from the time of the appearance of the tendril as an axillary papilla until it passed the sensitive stage. The greatest difficulty was encountered in fixing and hardening the material without causing the tendril to roll up in a helix, distorting the wall and contents of the cells. After a wide range of experiments, the best results were obtained by the use of acetic alcohol (1 part glacial acetic acid, 6 parts absolute alcohol [or 96 per cent], and 3 parts chloroform). In this killing agent the tendril, after two or three oscillations, regained and kept almost the original form.

MACDOUGAL described the tendrils of *Passiflora caerulea* as filamentous organs springing from the axils of the leaves. In tendrils 1-3 cm. in length the whole surface often had a reddish purple tinge, due to color bodies in the subepidermal cells. With growth the color became distributed in ill defined longitudinal bands. When a tendril had reached the coiling stage, three distinct regions were to be found, the base, or non-coiling part 3-4 cm.; the middle or coiling region, comprising the greater part of the organ; and the hooked tip 4-6 mm. in length. The whole organ showed a bilateral organization, which was least apparent at the base, and most pronounced in the portion having the greatest power of movement.

The basal portion of the tendril was broadly oval, slightly flattened on the concave side; the middle portion was oval, with the lateral diameter much greater than the transverse, and with the lower surface distinctly flattened; the tip was nearly circular in outline and bore at the extreme end a cup-shaped depression, the result of excessive growth in length of the periblem. Along the convex upper and the lateral sides of the tendril were obscure angles, which were usually absent from the concave side.

Sections through the middle portion of the tendril showed the following structure: The epidermis consisted of a layer of rectangular cells with stomata on both surfaces. The layer of collenchyma was found to have the tangential walls much thicker than the radial. At the obtuse angles this layer consisted of three cells; at other places it had one. In this tissue were found the color bodies. The parenchyma consisted of loosely arranged, thin-walled cells containing much chlorophyll and protoplasm in the outer row. The inner row of cells was heavily laden with starch. Crystals of calcium oxalate were found throughout the parenchyma. The cells of this layer on the convex side of the tendril were larger than on the concave surface. The intercellular spaces were large and many. In many cases, however, the entire ends of adjacent cells were pressed closely together, so that one cell sent a protrusion into the cavity of another. The structure and arrangement of these cells fitted them for great variation in size, while the intercellular spaces, affording space for the reception of expelled cell sap, made possible rapid changes in tension. The parenchyma was connected with the central pith by medullary rays 2-4 cells in height. The bast region consisted of thin-walled, closely packed cells containing much protoplasm. As the tendril approached maturity these cells became less active, and took on excessive wall thickenings. When the tendril was still very young, this tissue formed a continuous band interrupted only by the medullary rays. The cambium appeared about the time of maturity, and retained its activity even after coiling had taken place. The primary xylem elements were about ten; half in a nearly straight row across the concave side, and the remainder in a semicircle to conform to the outline of the convex side. Each bundle consisted of two or three

spiral vessels arranged radially. These vessels showed lignification even in the young tendril. A continuous band of wood was found on the concave side by the formation of the secondary bundles. The xylem elements of the convex side did not change until after coiling. The central pith was composed of large parenchyma cells containing protoplasm.

The basal portion of the tendril differed from the middle portion in its regular oval outline, symmetrical arrangement of the xylem, heavier thickening of the collenchyma, and the early formation of a continuous distinct cambium zone. Lignification had extended slightly to the pith and parenchyma in the xylem, which had three or four spiral vessels beside an annular vessel in each bundle. The central pith was usually torn apart. The structure of the tip differed widely from the base or middle region. The cup formation lay transversely to the length of the tendril near the extremity of the concave side. The epidermal cells were smaller toward the end of the tendril. The collenchyma was composed of one row of shortened, thickened cells terminating at the rim of the cup. The chlorophyll layer was reduced in size, and showed an increased density in protoplasm. The parenchyma layer was relatively very thick. The cells were angular and distorted. The bast and cambium disappeared after they entered this region. The bundles of the convex side and the band of the concave side were separated by a thin bundle of pith as they neared the tip. Pits were present in all the tissues of the tendril. The protoplasm was most dense and granular in the epidermal and chlorophyllous cells of the concave side near the tip. Density of protoplasm decreased in the middle region.

MACDOUGAL assumed in conclusion (1) that the concentration of the protoplasm in the epidermal layer had a direct connection with irritability; (2) that the movements of the organs were due to changes in the chlorophyll layer; (3) that the disposition of the xylem elements was favorable to rapid flexion and extension; and (4) that the abundant food supply reserve was a provision for the rapid growth and fixation of the tendril upon coiling.

Later MACDOUGAL (4) added a few points to the structure of *Passiflora* tendrils which were not given in the earlier paper.

He described the epidermal cells as rectangular in outline, with the long axis parallel to the long axis of the tendril. The protoplasts were found to occupy a large proportion of the cell cavity. The nucleus, especially on the concave side, usually lay against the inner wall. The structure of the protoplasm of the convex and concave sides was quite different, that of the concave side being more richly granular, and more nearly filling the cell cavity. The density of the protoplasm of the concave side increased from base to tip, apparently corresponding with the degree of irritability to contact. The outer walls of the concave side were slightly arched outwardly, and became cuticularized only after maturity. These convexities, MACDOUGAL suggested, must greatly increase the delicacy of perception of stimuli.

The epidermal layer contained stomata which communicated with the cortical parenchyma by very narrow intercellular passages. The collenchyma layer was one, two, or three cells in thickness. The protoplasm of the concave side almost completely filled the cells, and was densely granular, in marked contrast with that of the convex side. MACDOUGAL remarked that this difference between the contents of the collenchymatous tissue of the two sides was an indication that the densely granular condition of the concave side bore a connection with the transmission of impulses transversely or longitudinally. The granular contents, however, might be present merely as reserve substance.

In comparing the parenchyma cells internal to the collenchyma, MACDOUGAL found differences in their form and behavior in the convex and concave sides. The cells of the concave side were longer in proportion to their width than those of the convex side. The cells of the concave side had tapering ends, and the end walls were united over a small area only, thus forming large intercellular spaces. The cells of the convex side were united more completely across the ends, and the intercellular spaces were accordingly smaller. The protoplasm of the parenchyma cells also was more densely granular on the concave side, and more nearly filled the cell cavity. The parenchymatous cells were arranged in four or five layers on both the concave and convex sides of the tendril,

but those of the convex had the greater diameter, thus giving this side preponderance in thickness. In the two outer layers were chloroplasts varying from six to ten in number. The parenchymatous tissue of both cortex and pith exhibited marked infoldings of the walls. During curvature MACDOUGAL found that the cells of the parenchyma on the concave side decreased in size from 20 to 40 per cent of their original volume, and became irregularly globoid or ovoid. This MACDOUGAL thought to be explained by "an increase in the permeability of their protoplasts, a consequent extrusion of water into the intercellular spaces, and a release of the stretching tension of their walls, the elastic contraction of which caused the resultant curvature."

HABERLANDT (2) observed that in those tendrils which were sensitive on one side only, the sensitive side was usually without hairs and had few if any stomata. He noted that the sensitive epidermis of tendrils differed from other epidermis in various points. The epidermal cells were usually smaller but deeper. The outer walls were scarcely cutinized at first, but after the tendril had attached itself the walls became thick and heavily cutinized. The true cuticle was usually well developed and folded, although sometimes it was absent at the base of the tendril. The folding of the cuticle was much more pronounced in the sensitive portions of the tendrils of *Passiflorae* and others than it was on the stem and petiole of the same plants. He considered this folding of the cuticle to be an adaptation for the perception of stimulus. In most tendrils the outer walls of the sensitive epidermal cells curve outward, another useful adaptation. The epidermal cells were rich in cytoplasm, a characteristic of sense organs. HABERLANDT stated that PFEFFER (7) was the first to call attention to the sensitive papillae of *Cucumis sativus*, *Bryonia dioica*, and *Sicyos angulatus*. In *Cucumis* the papillae were observed only on the sensitive side of the tendril; in *Bryonia* they were found on both sides. In both plants, however, the papillae were almost completely absent from the basal, insensitive portion. PFEFFER called attention to the bowl-like widening of these points, so that the sensitive protoplasm was spread over an increased surface. As he did not find similar

papillae in all plants, he concluded that they were not absolutely essential for the perception of stimulus, but merely facilitated this perception.

HABERLANDT found great variety in the structure of these papillae. In *Cucurbita Pepo* the "sense epithelium" of the sensitive side lay above a continuous wide band of collenchyma. Stomata were absent, but a few club-shaped hairs were observed. The sense cells were somewhat stretched, the outer walls being $3-4\ \mu$ thick. On the center of each outer wall there was one papilla, with very rarely a second found near it. The interior of the papilla was widened in a funnel-shaped fashion, and was approximately circular in circumference, $1.5-6\ \mu$ in diameter. The canal leading from the inside of the papilla into the lumen of the cell was oval, or even slitlike in cross-section. The thin outer membrane of the papilla was flat, or sometimes very slightly bulged outward, and $0.6-0.8\ \mu$ in thickness. The thickness of the cuticle at this point was practically the same as at other regions in the tendril. Beneath it lay a very thin cellulose lamella. The sense cells contained a thick layer of protoplasm directly beneath the cell wall, in which were chromatophores containing starch, and a large nucleus against the inner wall. The papillae were usually completely filled with cytoplasm. If they were large, however, the cytoplasm seemed to lie against the wall. A crystal (probably calcium oxalate) usually lay in the protoplasm of the papilla. Sometimes the crystal was found in the opening of the canal, or in other positions. HABERLANDT offered no evidence as to the origin of this crystal (rarely more than one), but suggested that it bore a close relation to the perception of stimulus. A sudden pressure, bringing the sensitive membrane of the cell in contact with a corner of this crystal, might produce a stronger stimulus.

In *Lagenaria vulgaris*, as in *Cucurbita Pepo*, papillae were present only on the sensitive side. In shape the papillae much resembled those of *C. Pepo*. In the tendrils of *Cyclanthera explosens* the papillae were present only on the sensitive side. HABERLANDT described them here as being a "circular flat depression, easily overlooked." The outer wall of the cell was $1.5-2\ \mu$ in thickness, and the membrane over the papilla was $0.8\ \mu$ thick. The membrane showed a weak bowing outward.

BRUSH (1), in determining the influence of tension and contact upon the formation of mechanical tissue in the tendrils of *Passiflora caerulea*, used two methods of comparison, that is, their breaking strength and their anatomical structure. In studying the general anatomy of *Passiflora caerulea* tendrils, he found the following arrangement of tissues: epidermis, collenchyma, thin walled parenchyma, bast, xylem (which forms a complete ring due to secondary growth), and pith. In mature tendrils the pith filled the central part except in the basal portion of the tendril, where there was a central cavity.

At the base the arrangement of tissues was found to be very nearly radial, but in the "portion in contact" dorsiventrality was marked. A section halfway between base and apex showed a slight dorsiventrality, a somewhat greater amount of xylem being formed on the concave side.

The mechanical tissue of a free tendril, as shown by cross-sections through the middle of the tendril, was limited to a small area of xylem on the concave side, and the four primary cells were comparatively thin walled, and the primary bundles of the opposite side were composed of two or three slightly thickened vessels. Only a few bast fibers were present, and a very little pith, which lined a central cavity. Toward the base a complete ring of thin walled xylem and pith was formed.

A comparison of sections of tendrils under contact and under tension showed that while the xylem areas were approximately the same in both, in the tendril under tension the walls had become much thickened in the pith cells, while in the one under contact alone the pith cells were quite thin walled. This thickening of walls took place throughout the whole area of the pith in the tendril under tension. The pith was thin walled throughout in the tendril under contact alone. BRUSH noted that those tendrils under tension were harder to cut, owing no doubt to a difference in density of cell walls. An examination of sections of the middle third of tendrils with and without tension showed the diameter of mechanical tissue to be greater in the latter. BRUSH, having found in experiments on the breaking strength of tendrils that those under tension had a breaking strength nearly 50 per cent higher (average under tension 1239 gm., average tension-free 862 gm.), found the greater

area of mechanical tissue in the tendril grasping a support but not under tension somewhat surprising. He thinks the increased strength with tension partly accounted for by the thickening of the pith cell walls, this thickening usually extending to the center. Measurements with a planimeter showed the comparative areas to be as follows:

	XYLEM	PITH (thick walled)	TOTAL
Under tension.....	2.71	2.78	5.39
Tension free	4.51	2.78	4.51

BRUSH found that a marked characteristic of sections of tension free tendrils was that the pith became displaced in sectioning, while in those from tendrils under tension the pith held its shape as if firm.

Sections made from the basal part of tension-free tendrils and those under tension showed remarkably the transference of stimulus for growth through the tissues to the basal portion of the tendril. In the base of a tendril in which the middle third only had been under tension, the pith was thick walled throughout, while in the base of a tension-free tendril the pith was thin walled. BRUSH states that the marked increase in the thickness of pith walls which was found only when tension was present can be explained only by the theory that this thickening is due to the longitudinal pull on the tendril, by which the tensile strength of the tendril is increased.

Examination of sections through the contact region of tendrils which had been placed under pressure by a column of mercury failed to show any difference in anatomical structure when compared with tendrils in contact only. Sections through the middle of tendrils not under tension, but placed under pressure and under contact, were markedly different in their amount of xylem. The area of xylem in an average tendril under pressure was approximately twice as great as in a tendril which had been in contact only. The pith was thin walled in both sets of tendrils. BRUSH concludes from his anatomical studies on *Passiflora* "that contact pressure causes a greater formation of xylem in the tendril, while longitudinal tension causes a thickening of the walls of the pith whereby greater tensile strength is secured."

A study of the cellular structure of the tendrils of *Cyclanthera explodens* has been carried on by the writer. Thirty-three free,

uncoiled, and (so far as possible) unstimulated tendrils of *Cyclanthera explodens*, varying in length from 1 cm. to 12.6 cm., were studied cytologically from stained preparations. These tendrils were sectioned throughout their entire length. Freehand sections from living material also were studied.

Great difficulty was at first experienced in fixing those tendrils which had reached the sensitive stage, but their reaction to the killing agent was practically overcome, in the great majority of cases, by inserting the tendrils in glass tubes of small bore immersed in the solution. Fleming, weak, medium, and strong, and Picro formin were the killing agents used. Triple Fleming was used in staining.

Cross-sections of the basal portion of a sensitive tendril (a tendril of at least 6 cm. in length) were markedly radial in structure. Six well defined lobes were frequently observed, four large and two very much smaller. One or both of the latter were often missing, but if present were found on the dorsal surface of the tendril. The number of lobes always corresponded to the number of bundles found in this region. The epidermis was protected by a thick cutin, which rendered the epidermal cells difficult to cut without becoming torn. The epidermal cells were rectangular in shape, with their depth greater than their width. The outer walls curved outward in all cases, and were frequently extended to form "papillae." These papillae were more numerous, however, in the basal portion of the tendril than in the sensitive region. They were usually found upon the outermost projection of the lobes, one to three in number, with often two papillae lying in adjacent cells. The "sense cells" were more narrow than the ordinary epidermal cell, and, as HABERLANDT stated, had large nuclei which lay on or near the inner wall. The cytoplasm extended into the papilla, usually filling it.

The epidermis of this region, as of all other portions of both sensitive and insensitive tendrils, was found to be more or less plentifully provided with glandular structures, usually club-shaped, but with all variations in size and shape. These epidermal outgrowths were sometimes found as an enlarged papilla-like structure into which the cell contents had entered. This cell often divided,

and the process of division continued until the filament was three or four cells in length (fig. 1). In other instances, the outgrowth was plainly separated from the epidermal cell by the cell wall. Crystals were common in these bodies, especially after the connection with the tendril had disappeared. Crystals were also found in the parenchyma of the tendril. The collenchyma of the basal region was found only in the angles of the lobes. This layer was composed of 3-5 rows of cells having the tangential walls heavier than the radial. These rows of cells were usually deeper in the ventral, and right and left lobes than upon the dorsal surface. Frequently the inner row appeared to be a transition between collenchyma and parenchyma. The cortical parenchyma was 2 or 3 rows in depth, the inner row being composed of larger cells, and containing starch in abundance. The starch content was especially heavy in those cells lying in the outermost projection of the lobes, and at the lowest point in the curves between the lobes. The closed ring of bast, composed of very much elongated cells, densely filled with cytoplasm, conformed in shape to the outline of the cross-section of the tendril. The bundles, as before stated, corresponded in number to the lobes. The largest bundle lay on the ventral lobe. The pith parenchyma consisted of large, thin walled cells, very irregular in size and shape, but closely interlocked with one another. The intercellular spaces were small.

A cross-section through the middle portion of any tendril showed it to be dorsiventral in structure. The large lobe of the dorsal surface had given place to an indentation on this side (fig. 2). The bast layer now extended across the concave side only, with two small strips lying outside the small bundles of the convex (dorsal) surface. The number of bundles throughout the dorsiventral portion of a tendril was commonly five. An exception to this was noted in a specimen in which the branch and main axis of the tendril were united until within a short distance of the tip. Here the bundles were eight in number, although the structure was dorsiventral.

Gradually the central lobe of the concave side disappeared, and the outline of this surface was found to be broadly semicircular in the sensitive region of a tendril (fig. 3). The collenchyma layer of the concave side in this region consisted of apparently one row

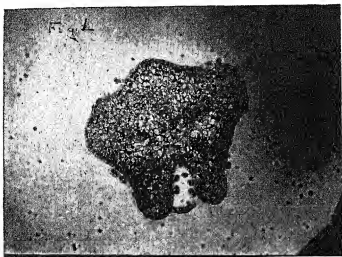


FIG. 1

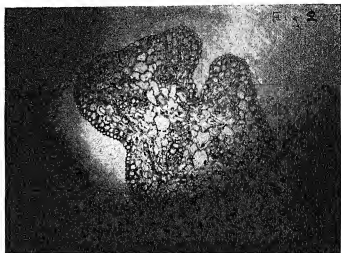


FIG. 2

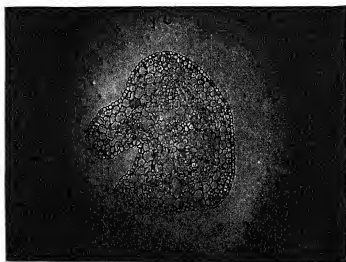


FIG. 3

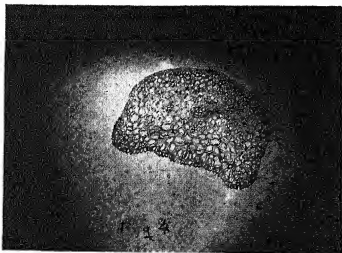


FIG. 4

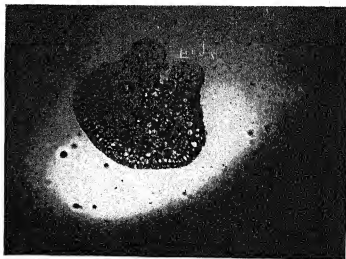


FIG. 5

of cells, whose walls showed a tendency to break down, often throwing four or five cells together. This was much more marked upon the concave side than on the convex, but, as this breaking down had been observed in a slight degree in the basal portion of the tendril, it was of doubtful significance. The parenchyma cells of the convex side were now seen to be much larger than those of the concave. Both cortical and pith parenchyma contained large intercellular spaces.

The indentation upon the convex side now widened until a cross-section nearing the tip of the tendril became semicircular, or almost rectangular in outline (fig. 4). The collenchyma of the concave side consisted of three rows of cells, the outer row being of very small cells of practically the same size and appearance as the epidermal cells. The pith parenchyma had now become greatly compressed, the bundles filling the greater part of the central space. A cross-section near the base of a young insensitive tendril (2-5 cm. in length) was found to be dorsiventral (figs. 1-5). The chief differences in structure which distinguished the young tendril from the upper portion of one which had reached the sensitive stage were the absence of strengthening tissue, the greater density of the cytoplasm, the presence of starch in greater quantity, and the large number and size of the nuclei.

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NITROGEN CONTENT OF WEEDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 321

E. G. CAMPBELL

(WITH FOUR FIGURES)

Several years ago Woo¹ made a chemical analysis of *Amaranthus retroflexus*. His work included these three stages of development: (1) young plants (collected June 5, one-fourth inch in height); (2) plants just before blooming (collected July 8, 20 inches in height); and (3) an intervening age (collected June 20, 6-8 inches in height). The purpose of his work in part was to investigate the claim, often made, that *Amaranthus* has the power to store nitrates. He found the largest percentage of total and nitrate nitrogen in the stage of growth just before blooming, but was unable to extend his investigation through the entire season, to include mature plants. In 1920 the writer, under the direction of Dr. WILLIAM CROCKER, undertook to investigate the nitrate nitrogen situation in *A. retroflexus*, including two ages, just before blooming and at maturity. These two growth periods were selected so as to check the work done by Woo and extend the investigation to the mature plant. Later the work was extended through 1921 and 1922, to include, in addition to *A. retroflexus*, twenty-five other weed species collected in three stages of development and under various conditions of growth.

Methods and results

In collecting specimens, care was taken that the three desired ages of any given species were secured from the same plot of soil, so as to insure so far as possible like conditions for the three stages of growth. The specimens were carefully cleaned in running tap water, oven dried, and ground to a fine powder. The powder was then brought to a constant dry weight, 2 gm. then being taken for the total nitrogen and .10 gm. for the nitrate nitrogen determination.

¹ Woo, M. L., Chemical constituents of *Amaranthus retroflexus*. BOT. GAZ. 68:313-344. 1919.

In all cases duplicate samples were run. In the work of 1920 and 1921 analyses were made of the separate parts of each specimen (root, stem, leaves, and fruit), but in the work of 1922 the entire plant of each growth stage was analyzed. The fall and winter conditions of several species were investigated and compared. In the case of *Amaranthus retroflexus*, specimens growing under abnormal conditions (on manure piles and dumps of hog hair) as well as field specimens were analyzed.

The Kjeldahl-Gunning method was followed in finding the percentage of total nitrogen. For the nitrate nitrogen determina-

TABLE I
NITRATE NITROGEN IN THREE AGES OF *Amaranthus retroflexus* AS
DETERMINED BY TWO METHODS

STAGE	PART	SCHLÖSING-WAGNER METHOD		PART	PHENOLDISULPHONIC ACID METHOD	
		Nitrate nitrogen			Nitrate nitrogen	
		I	II		I	II
Young.....	Root.....	0.74	0.78	Root.....	0.64	0.59
	Stem.....	0.84	0.86	Stem.....	0.75	0.78
	Leaves.....	0.64	0.65	Leaves.....	0.45	0.48
Just before blooming..	Root.....	1.02	0.99	Root.....	0.92	0.95
	Stem.....	1.84	1.89	Stem.....	1.40	1.45
	Leaves.....	Trace		Leaves.....	0.25	0.28
Mature.....	Root.....	Trace		Root.....	0.00	0.00
	Stem.....	Trace		Stem.....	0.00	0.00
	Leaves.....	0.00	0.00	Leaves.....	0.00	0.00
	Fruit.....	0.00	0.00	Fruit.....	0.00	0.00

tion in *A. retroflexus*, both the Schlösing-Wagner volumetric method as modified by KOCH, and the phenoldisulphonic acid colorimetric method were employed. In all other species involved, the nitrates were determined by the phenoldisulphonic acid method alone. After paralleling the two methods in a great number of test analyses, the writer prefers the latter, because the apparatus involved is more easily and accurately operated. Of the two methods employed (volumetric and colorimetric) in the nitrate nitrogen determination on *A. retroflexus*, a higher percentage was repeatedly obtained by the volumetric method. This was the one used exclusively by Woo, who found most nitrate nitrogen in

the stage of growth designated in this paper as just before blooming. So far as the writer used the volumetric method, he obtained results closely approximating and consistently paralleling those of

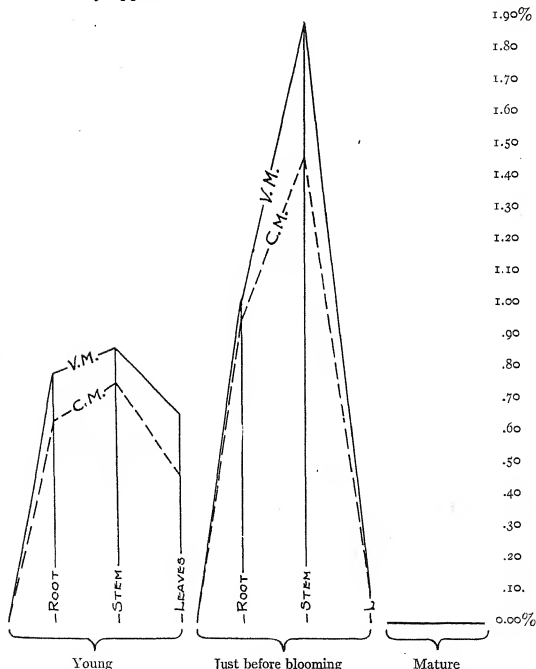


FIG. 1.—Nitrate nitrogen in *Amaranthus retroflexus*, in three stages of development grown under field conditions, as determined by two methods: V.M., volumetric (Schlösing-Wagner); C.M., colorimetric (phenoldisulphonic).

Woo. The results reached through the colorimetric method, however, ran slightly and persistently lower, as shown in table I and fig. 1.

The work of 1920 included only four species, *A. retroflexus* in two growth stages (just before blooming and mature), and the

TABLE II

NITRATE NITROGEN; VOLUMETRIC METHOD, 1920

Stage	Part	I	II
Amaranthus retroflexus (just before blooming) . . .	Root.....	0.43	0.43
	Stem.....	0.31	0.30
	Leaves.....	0.19	0.18
Amaranthus retroflexus (mature).....	Root.....	0.00	0.00
	Stem.....	Trace
	Leaves.....	0.00	0.00
	Fruit.....	0.00	0.00
Abutilon Theophrasti (mature).....	Root.....	0.00	0.00
	Stem.....	0.00	0.00
Erigeron canadensis (mature).....	Root.....	0.00	0.00
	Stem.....	0.00	0.00
Xanthium italicum (mature).....	Root.....	0.00	0.00
	Stem.....	0.00	0.00

TABLE III

RATIO BETWEEN TOTAL NITROGEN AND NITRATE NITROGEN IN THREE AGES OF *Amaranthus retroflexus*

STAGE	PART	TOTAL NITROGEN		NITRATE NITROGEN	
		I	II	I	II
Young.....	Root.....	3.96	3.96	0.64	0.59
	Stem.....	2.92	2.94	0.75	0.78
	Leaves.....	4.63	4.53	0.45	0.48
Just before blooming..	Root.....	3.12	3.90	0.92	0.95
	Stem.....	3.58	3.71	1.40	1.45
	Leaves.....	4.59	4.55	0.25	0.28
Mature.....	Root.....	0.63	0.67	0.00	0.00
	Stem.....	0.24	0.28	0.00	0.00
	Leaves.....	1.99	2.00	0.00	0.00
	Fruit.....	2.58	2.66	0.00	0.00

root and stem of *Abutilon Theophrasti*, *Erigeron canadensis*, and *Xanthium italicum* in the mature stage only. None of these four species contained nitrates at maturity except *A. retroflexus*, which showed a trace in the stem (table II). In the stage just before

TABLE IV

STAGE	PART	TOTAL NITROGEN		NITRATE NITROGEN	
		I	II	I	II
Chenopodium album					
Young.....	{Root.....	1.18	1.21	0.10	0.09
	{Stem.....	2.56	2.57	0.28	0.24
	{Leaves.....	5.09	5.12	0.08	0.12
Just before blooming.....	{Root.....	0.89	0.95	0.32	0.32
	{Stem.....	1.27	1.29	0.10	0.09
	{Leaves.....	4.63	4.69	Trace
Mature.....	{Root.....	0.52	0.54	0.00	0.00
	{Stem.....	0.63	0.67	0.00	0.00
	{Leaves.....	3.96	3.96	0.00	0.00
	{Fruit.....	3.11	3.15	0.00	0.00
Xanthium italicum					
Young.....	{Root.....	0.81	0.77	0.16	0.15
	{Stem.....	1.10	1.06	0.20	0.21
	{Leaves.....	4.52	4.48	0.20	0.24
Just before blooming.....	{Root.....	1.54	1.54	0.12	0.14
	{Stem.....	2.19	2.20	0.25	0.27
	{Leaves.....	4.50	4.48	0.12	0.14
Mature.....	{Root.....	0.70	0.68	0.00	0.00
	{Stem.....	0.29	0.29	0.00	0.00
	{Leaves.....	1.96	1.96	0.00	0.00
	{Fruit.....	1.79	1.79	0.00	0.00
Lactuca scariola					
Young.....	{Root.....	1.54	1.54	Trace
	{Stemless.....				
	{Leaves.....	3.88	4.00	Trace
Just before blooming.....	{Root.....	0.77	0.70	Trace
	{Stem.....	0.77	0.79	Trace
	{Leaves.....	3.35	3.31	Trace
Mature.....	{Root.....	0.51	0.52	0.00	0.00
	{Stem.....	0.35	0.37	0.00	0.00
	{Leaves.....	1.37	1.40	0.00	0.00
	{Fruit.....	2.45	2.43	0.00	0.00
Ambrosia artemisiifolia					
Young.....	{Root.....	0.96	0.96	Trace
	{Stem.....	1.59	1.59	0.12	0.14
	{Leaves.....	4.56	4.53	0.00	0.00
Just before blooming.....	{Root.....	0.50	0.50	0.00	0.00
	{Stem.....	0.57	0.57	0.00	0.00
	{Leaves.....	3.59	3.37	0.00	0.00
Mature.....	{Root.....	0.35	0.32	0.00	0.00
	{Stem.....	0.25	0.27	0.00	0.00
	{Leaves.....	2.10	2.14	0.00	0.00
	{Fruit.....	2.06	2.04	0.00	0.00

TABLE IV—Continued

STAGE	PART	TOTAL NITROGEN		NITRATE NITROGEN	
		I	II	I	II
Erigeron canadensis Young.....	{Root.....	0.61	0.69	0.00	0.00
	{Stem.....	0.98	1.06	0.00	0.00
	{Leaves.....	3.43	0.00	0.00
Just before blooming.....	{Root.....	0.35	0.00	0.00
	{Stem.....	0.38	0.35	0.00	0.00
	{Leaves.....	3.43	3.45	0.00	0.00
Mature.....	{Root.....	0.29	0.29	0.00	0.00
	{Stem.....	0.35	0.35	0.00	0.00
	{Leaves.....	2.65	2.67	0.00	0.00
	{Fruit.....	1.95	2.07	0.00	0.00
Abutilon Theophrasti Young.....	{Root.....	0.77	0.75	0.00	0.00
	{Stem.....	0.69	0.71	0.00	0.00
	{Leaves.....	3.22	3.20	0.00	0.00
Just before blooming.....	{Root.....	0.63	0.67	0.00	0.00
	{Stem.....	0.41	0.37	0.00	0.00
	{Leaves.....	2.56	2.52	0.00	0.00
Mature.....	{Root.....	0.32	0.32	0.00	0.00
	{Stem.....	0.36	0.39	0.00	0.00
	{Leaves.....	1.67	1.65	0.00	0.00
	{Fruit.....	2.26	2.26	0.00	0.00

blooming the results reached closely approximate Woo's figures for root, stem, and leaves.

In the work of 1921, *Amaranthus* in three stages of its development (young, just before blooming, and mature) was analyzed for both total and nitrate nitrogen (table III).

During the same season six other species were tested with *Amaranthus retroflexus*, namely, *Chenopodium album*, *Xanthium italicum*, *Lactuca scariola*, *Ambrosia artemisiifolia*, *Erigeron canadensis*, and *Abutilon Theophrasti*. The results obtained in these additional species are shown in table IV.

The work of 1922 includes *Amaranthus retroflexus* growing under abnormal conditions, and eighteen other species of weeds collected in three stages of development and under various conditions of growth. The species are as follows: *Rumex crispus*, *Erigeron annuus*, *Potentilla monspeliensis*, *Capsella Bursa-pastoris*, *Veronica*

peregrina, *Amaranthus blitoides*, *A. graecizans*, *Atriplex patula*, *Salsola Kali*, *Polygonum pennsylvanicum*, *P. convolvulus*, *Portulaca oleracea*, *Daucus carota*, *Arctium minus*, *Solanum carolinense*, *Cirsium arvense*, *Physalis heterophylla*, and *Asclepias syriaca*. In this group of plants no attempt was made to divide the specimens into root, stem, leaves, and fruit; the whole plant of each growth stage was analyzed.

In the spring of 1922, rye and five contemporary winter annual weeds were compared in three stages of growth. Rye was collected

TABLE V

WEEDS GROWING WITH RYE, COLLECTED IN THREE GROWTH PERIODS

I WHEN RYE WAS 2-3 INCHES HIGH

II WHEN RYE WAS BEGINNING TO JOINT

III WHEN RYE WAS IN FULL HEAD

Plant and growth period	Total nitrogen	Nitrate nitrogen	Plant and growth period	Total nitrogen	Nitrate nitrogen
Rye			Veronica peregrina		
I.....	3.78	0.08	I.....	2.38	0.04
II.....	2.11	0.05	II.....	1.40	0.04
III.....	1.19	0.02	III.....	1.26	0.04
Rumex crispus			Erigeron annuus		
I.....	3.22	0.10	I.....	3.36	0.08
II.....	2.38	0.04	II.....	1.68	0.04
III.....	1.96	0.04	III.....	1.13	0.04
Capsella Bursa-pastoris			Lactuca scariola		
I.....	4.62	0.15	I.....	3.36	0.08
II.....	3.05	0.08	II.....	1.96	0.04
III.....	1.54	0.02	III.....	1.78	0.04
Potentilla monspeliensis					
I.....	3.36	0.12			
II.....	1.68	0.04			
III.....	1.14	0.04			

when from two to three inches high, when beginning to joint, and when in about full head. *Capsella Bursa-pastoris*, *Erigeron annuus*, *Lactuca scariola*, *Potentilla monspeliensis*, and *Rumex crispus* were each collected on the same days and in the corresponding stages of growth as the five rye collections. The results of this investigation are shown in table V. It is interesting to note that there is a close parallel between rye and weeds, both in total and nitrate nitrogen, and that throughout the series the curve goes

TABLE VI

Stage	Percentage total nitrogen	Percentage nitrate nitrogen	Lb. of nitrate in soils per acre
<i>Polygonum pennsylvanicum</i>			
Young.....	1.42	Trace
Just before blooming.....	3.38	0.1	3
Early maturity.....	0.55	0.00	8
<i>Polygonum convolvulus</i>			
Young.....	3.57	0.06
Just before blooming.....	3.58	0.06	8
Early maturity.....	0.63	0.00	5
<i>Chenopodium album</i>			
Young.....	3.78	0.04
Just before blooming.....	3.50	0.16	70
Early maturity.....	0.92	Trace	8
<i>Salsola Kali</i>			
Young.....	2.66	Trace
Just before blooming.....	3.16	0.06	3
Early maturity.....	1.19	0.00	2
<i>Amaranthus blitoides</i>			
Young.....	3.50	0.10
Just before blooming.....	3.69	0.27	34
Early maturity.....	2.10	Trace	24
<i>Amaranthus graecizans</i>			
Young.....	4.31	0.15
Just before blooming.....	4.00	0.56	26
Early maturity.....	1.01	Trace	22
<i>Portulaca oleracea</i>			
Young.....	4.48	0.26
Just before blooming.....	2.85	0.12	26
Early maturity.....	1.51	Trace	7

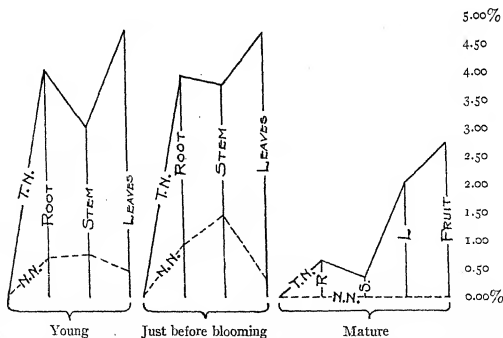


FIG. 2.—Ratio between total nitrogen (T.N.) and nitrate nitrogen (N.N.) in *Amaranthus retroflexus* in three stages of growth, taken under field conditions.

progressively lower from young to mature stages. *Capsella Bursa-pastoris* leads all other species in this series in the first two stages of growth, both in total and nitrate nitrogen.

Soil samples were taken at the roots of seven species just before blooming and at maturity, and tested for nitrate nitrogen, which was calculated in pounds per acre (2), as shown in table VI.

The third growth stage of the plants used in table VI is early rather than full maturity, as in all other cases. In this stage a

TABLE VII

Stage	Part	Percentage total nitrogen	Percentage nitrate nitrogen	Lb. of nitrate in soil per acre
<i>Atriplex patula</i> (on well decayed hog hair)				
Young.....		3.46	0.22
Just before blooming.....		3.70	0.46	60
Early maturity.....		1.87	0.12	9
<i>Amaranthus retroflexus</i> (on decayed hog hair)				
Young.....		4.50	0.65
Just before blooming.....		4.55	0.52	140
Early maturity.....		1.47	0.08	75
<i>Amaranthus retroflexus</i> (in edge of horse stable)				
Young.....		4.97	0.36
Just before blooming.....		4.62	0.56	650
Early maturity.....		2.60	0.25	1175
<i>Amaranthus retroflexus</i> (on manure pile)				
Young.....	{Root.....	2.21	Trace
	{Stem.....	2.42	Trace
	{Leaves.....	4.06	0.00
Just before blooming.....	{Root.....	2.70	0.38
	{Stem.....	3.47	0.56
	{Leaves.....	4.29	0.10
Mature.....	{Root.....	1.59	0.40
	{Stem.....	1.41	0.45
	{Leaves.....	3.75	Trace
	{Fruit.....	3.92	Trace

trace of nitrate was usually found, whereas at full maturity none was found.

By neither method employed was nitrate nitrogen found in a fully matured specimen of *Amaranthus retroflexus*, grown under field conditions, nor in any of the other twenty-four species tested. The nitrate curve rises gradually from the early seedling stage to the blooming stage, where it begins its decline, reaching zero at full maturity (fig. 2). Nearly all the annuals tested in early maturity

showed at least a trace of nitrate. This regular nitrate curve was not obtained in the case of plants grown under abnormal conditions. Table VII gives the results obtained on *A. retroflexus* for two successive years, in part, and *Atriplex patula* for one year. In these specimens the nitrate was not reduced to a trace at early maturity, nor was the zero mark reached at full maturity.

TABLE VIII

Plant	Season	Percentage total nitrogen	Percentage nitrate nitrogen
Capsella Bursa-pastoris..	{ Spring	4.62	0.15
	{ Fall	4.20	0.12
Erigeron annuus	{ Spring	3.36	0.12
	{ Fall	2.10	0.04
Lactuca scariola	{ Spring	3.36	0.08
	{ Fall	3.60	0.10
Potentilla monspeliensis..	{ Spring	3.36	0.12
	{ Fall	3.50	0.08
Daucus carota	{ Spring	3.68	0.12
	{ Fall	2.29	0.04
Arctium minus	{ Spring	2.78	0.13
	{ Fall	2.15	0.08
Rumex crispus	{ Spring	3.22	0.10
	{ Fall	2.45	0.04
Solanum carolinense....	{ Spring	3.12	0.12
	{ Fall	1.75	0.00
Cirsium arvense	{ Spring	4.27	0.20
	{ Fall	1.68	0.10
Asclepias syriaca	{ Spring
	{ Fall	1.35	0.00
Physalis heterophylla....	{ Spring
	{ Fall	1.82	0.00

Specimens of certain species of winter annuals and biennials were collected in the fall, and compared as to nitrogen content with specimens of the same species taken in the spring. It was found for most species that the percentages were higher in the spring (table VIII).

Those perennials tested whose above-ground portions mature and die at or before the first frost contain no nitrate at the beginning of winter, namely, *Solanum carolinense*, *Asclepias syriaca*, and *Physalis heterophylla*. The perennials whose lower leaves remain green after frost, however, as *Cirsium arvense*, *Rumex crispus*, etc.,

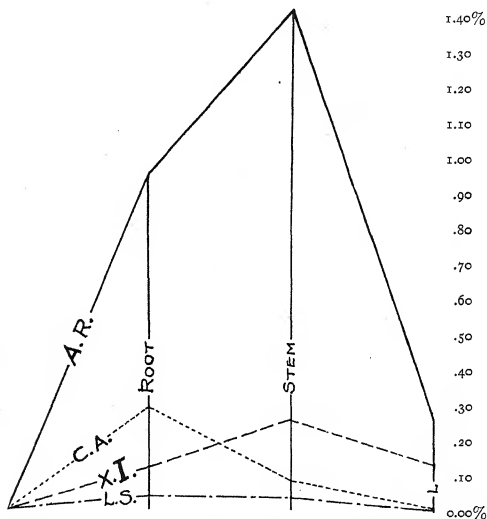


FIG. 3.—Percentage relations of nitrate nitrogen in *Amaranthus retroflexus* (A.R.), *Xanthium italicum* (X.I.), *Chenopodium album* (C.A.), and *Lactuca scariola* (L.S.), taken under field conditions just before blooming.

not only have some nitrate nitrogen in early winter, but contain a larger portion of total nitrogen than do those perennial species whose aerial portions die at frost. It has been suggested that the ability of *Amaranthus retroflexus* to absorb great quantities of nitrates might be an inherited trait, and that other species of the same and

possibly the preceding and succeeding alliances may have the same tendencies.

In the analyses of 1920 and 1921 only four species showed any appreciable and consistent amount of nitrate nitrogen, namely, *Amaranthus retroflexus*, *Chenopodium album*, *Xanthium italicum*,

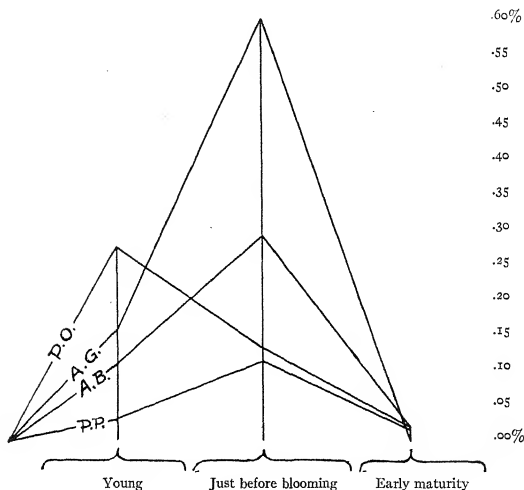


FIG. 4.—Nitrate nitrogen in *Amaranthus graecizans* (A.G.), *A. blitoides* (A.B.), *Portulaca oleracea* (P.O.), and *Polygonum pennsylvanicum* (P.P.), taken under field conditions in three stages of development.

and *Lactuca scariola*. The nitrate ratio of these species is shown in fig. 3. Of the additional species tested in 1922, it was found that the four plants leading in nitrate nitrogen content involved only Chenopodiales and its two adjacent alliances, Polygonales and Caryophyllales, with Chenopodiales again leading. Table VI shows the analyses of species ranging through Polygonales, Cheno-

podiales, and Caryophyllales. Chenopodiales, the alliance in which the pigweeds are found, leads the preceding and succeeding alliances in nitrate content. Figures taken from the various tables of this paper, representing plant alliances ranging from Graminales to Campanales, indicate a slight but consistent lead for Chenopodiales in regard to nitrate nitrogen absorption. Fig. 4 shows the percentage relations of nitrate in *Amaranthus graecizans*, *A. blitoides*, *Portulaca oleracea*, and *Polygonum pennsylvanicum*. Here again *Amaranthus* is a strong leader. Of the twenty-one genera involved in the analyses of this paper, under no conditions has *Amaranthus* been surpassed. In plants growing under normal conditions, the largest amount of nitrate nitrogen was invariably found in that stage of growth just before blooming, the lowest percentage in early maturity, zero being reached at full maturity.

Summary

1. Nitrate nitrogen, prominent in young plants of *Amaranthus retroflexus* and some other weeds, during young and immature stages, completely disappears at full maturity, contrary to Woo's prediction. Twenty-five species of weeds have been tested, and none shows nitrate nitrogen in the mature stage.

2. In all species grown under normal conditions, the highest percentage of nitrate is found in that stage of development designated as just before blooming.

3. *Amaranthus retroflexus* and *Atriplex patula*, grown upon manure piles and heaps of decaying hog hair, vary from what seems to be the normal course in nitrate absorption. In these cases early maturity shows more than a trace, and at full maturity nitrate is still present.

4. Of the two methods followed in nitrate determination, the colorimetric was found to have advantages over the volumetric, in its ease of operation and certainty of results.

The writer is grateful to Dr. WILLIAM CROCKER, Dr. CHARLES A. SHULL, Dr. R. H. CARR, and Mr. P. H. BREWER for valuable suggestions.

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CORRELATION OF FOSSIL FLORAS OF RHODE ISLAND AND NEW BRUNSWICK¹

EDA M. ROUND

(WITH FOUR FIGURES)

The geographical positions of Rhode Island and New Brunswick make a comparative study of their fossil plants desirable. Such study is facilitated by recent work on the Fern Ledges flora by STOPES (5), in which about forty species from St. John, New Brunswick, have been described. Several of these may be cited as

TABLE I

SPECIES	LOCALITIES IN NARRAGANSETT BASIN										
	Pawtucket	Central Falls	Valley Falls	Lonsdale	Cranston	Arlington	Tunnel Providence	Silver Spring	Bristol	Portsmouth	Newport Mansfield, Mass.
1. <i>Calamites</i> sp.	*	*
2. <i>Calamites</i> Suckowi	*	..	*	..	*
3. <i>Annularia sphenophylloides</i> ..	*	..	*	*	*	*	..	*	*
4. <i>Annularia stellata</i>	*	..	*	*
5. <i>Sphenopteris valida</i>	*	*	..
6. <i>Oligocarpia splendens</i>	*	*	..
7. <i>Pecopteris Miltoni</i>	*	..	*
8. <i>Pecopteris plumosa</i>	*	*
9. <i>Neuropteris heterophylla</i>	*	..	*	*	*	..	*	..	*	*	..
10. <i>Cordaites</i> cf. <i>Robbii</i>	*

belonging to the Rhode Island coal. They include some of the most common and widespread species in the state, the names and localities of which are given in table I.

If the species listed are considered separately, certain facts become apparent.

1. *Calamites* sp. is a closely striated specimen with the same general size and internodal distance characteristic of the Canadian form. It is represented in Rhode Island both as fossil and cast, but, being indeterminable, is of slight correlative value.

¹Work started in partial fulfilment of requirement for the degree of Ph.D. at Brown University.

2. *Calamites Suckowi* Brgt. has been reported from all parts of the Upper Carboniferous in North America, although most characteristic of the Allegheny and Conemaugh formations. These horizons may be designated as Upper Westphalian and Lower Stephanian respectively, where correlation with Europe is desired (4).

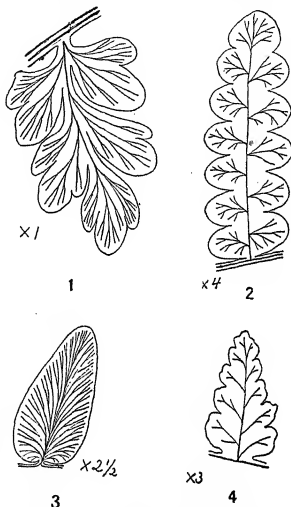
3. *Annularia spheno-phyloides* Gutbier, while appearing in all horizons of the Pennsylvanian period, seems to have been most widespread during the Conemaugh and Monongahela divisions thereof.

4. *Annularia stellata* Wood appears to have been most characteristic of the Allegheny and Conemaugh. Although it is common in the Cherokee shales of Missouri, a horizon regarded as lower productive coal (6), JONGMANS (2) regards it as typical of the upper part of the Upper Carboniferous, an opinion based largely on European conditions.

5. *Sphenopteris valida* Daws. (fig. 1) is regarded in Europe as a characteristic Westphalian species (5).

6. *Oligocarpia splendens* Daws. (fig. 4) is common to the Valen-ciennes flora of France, which is called Westphalian in horizon (7).

7. *Pecopteris Miltoni* Brgt. (fig. 2) appears to have been abundant in the lower coal of Europe (1), and so is useful as an index fossil of that period.



FIGS. 1-4.—Fig. 1, *Sphenopteris valida* Daws.: detail showing veining; drawn from specimen no. 868, Brown University coll.: fig. 2, *Pecopteris Miltoni* Artis: enlarged detail, $\times 4$; drawn from specimen no. 157, Brown University coll.: fig. 3, *Neuropteris heterophylla* Brongn.: enlarged detail, $\times 2.5$; drawn from specimen no. 354, Brown University coll.: fig. 4, *Oligocarpia splendens* Daws. sp.: enlarged detail, $\times 3$; drawn from specimen no. 315, Brown University coll.

8. *Pecopteris plumosa* Artis sp. is occasionally found in the Narragansett Basin, being apparently a narrow, simple form of *Pecopteris dentata* Brgt. STOPES states that "the species is widely distributed in the Westphalian and is particularly common in the Middle Coal Measures of Britain" (5).

9. *Neuropteris heterophylla* Brgt. (fig. 3), which is very common in the principal fossiliferous sections of Rhode Island, is interesting to botanists because of the fact that seeds have been found attached thereto (3). This characteristic shows the plant to have been a pteridosperm. It is the first supposedly fern type known to have produced seeds, thus furnishing an evolutionary link between the pteridophytes of the Carboniferous and modern flowering plants. While its vertical range extends from the Pottsville to the Dunkard formations, *Neuropteris heterophylla* is most characteristic of the Westphalian series in Europe.

10. *Cordaites* cf. *Robbii* Daws. is regarded as probably synonymous with *Cordaites borassifolius* Sternb., a common Rhode Island species. This genus represents the only group of gymnosperms from the coal shales of the Narragansett Basin.

While the species of fossil plants common to Fern Ledges and Rhode Island at present are too few for detailed correlation, the similarities between the localities are worthy of consideration, and should be helpful in making knowledge of the Carboniferous formations of Rhode Island more complete.

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THREE NEW SPECIES OF BENGAL POLYPORACEAE

S. R. BOSE

(WITH THREE FIGURES)

Polyporus Guhae, n.sp.—Pileus almost sessile or with short stalk, about 2 mm. thick, very hard, somewhat fan-shaped or petaloid, 2.4×2.5 –3 cm.: upper surface smooth, dull or dark brown, distinctly zoned, with raised ridges: hymenial surface blackish, faint yellow (with a brown tinge), shining; pores round, very minute, pore tubes longer than the context, which is very thin: margin with a black line, slightly involute: setae none: spores not found.

In imbricate clusters on rotten wood amongst grass, in interior of Howrah District, July 1916.

This species belongs to section *Petaloides* 15 of LLOYD's paper on "Stipitate Polyporoids" (thin, rigid Polypores, pale rose or reddish brown). LLOYD remarks: "We would enter this in section 15 of our pamphlet; on account of its rigidity and hardness it might be sought in *Trametes*, but the different color of pores and the context forbid."

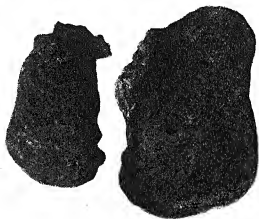


FIG. 1.—*Polyporus Guhae*, upper and lower surfaces.

Trametes Karii, sp. nov.—Pileus almost sessile, with short lateral stalk, somewhat reniform, soft, becoming somewhat coriaceous in drying, 7×4.5 cm.; also some very small forms, about 6 mm. thick, pale internally: upper surface glabrous, yellowish brown, a few faint rings toward the margin: hymenial surface dark brown; pores small, mostly round, pore tubes 3–4 mm. deep: margin entire, with a thin black line: setae none: spores not found.

In imbricate groups of three or four on dead wood amongst grass, Darjeeling, July 1918; Coimbatore, Madras, September 1921. Named for the late Dr. R. G. KAR, one of the founders of the Carmichael Medical College.

LLOYD remarks: "The plant impresses me as probably a white plant in its prime, being spotted brown when old or in drying, particularly when bruised. I should put it in section 128 of my journal, although it suggests the white plants in section 124."

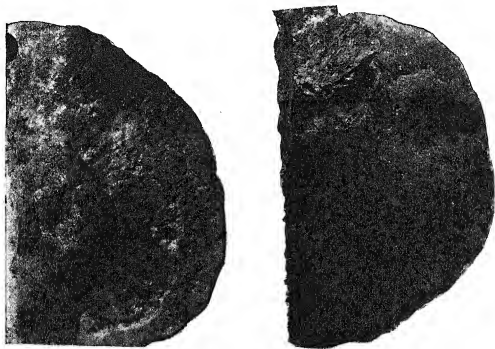


FIG. 2.—*Trameles Karii*, upper and lower surfaces

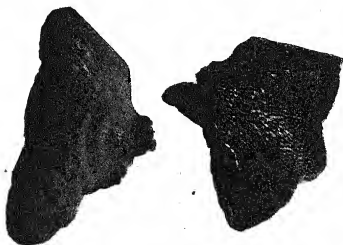


FIG. 3.—*Favolus Bengala*, upper and lower surfaces

Favolus Bengala, sp. nov.—Pileus almost sessile or with short lateral stock, about 2 mm. thick, coriaceous, 2-4 cm. long, 2-3 cm. broad, tapering toward the base, expanded at the margin: upper

surface perfectly smooth, dull white, marked by faint concentric rings: hymenial surface with pores fairly large, elongated, somewhat irregular and crenate; pore mouths yellowish, pore tubes deep, 2-5 mm. long, context very small: marginal outline wavy, sometimes broken up into corners: setae none: spores very few and rare, globose, $4\ \mu$ in diameter, almost hyaline.

On dead wood, in imbricate groups, interior of Hugli District, Bengal, August 1916.

LLOYD remarks: "This is an ambiguous *Favolus*, but we think it should be so classed on account of its large elongated pores, which are arranged in lines, indicating its affinities to a lamelliform arrangement. The rigid, firm texture of the plant, however, suggests *Trametes*."

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BRIEFER ARTICLES

A METHOD OF SECURING SPORES OF YEAST

One of the methods generally recommended for obtaining endospores of yeast is to grow the yeast on sterile blocks of plaster of Paris, a method perfected by HANSEN¹ a number of years ago. During the spring of 1923 the writer discovered that the surface layers of cakes of Fleischmann's yeast which had been kept in an ice chest in the laboratory for several (6-8) days contained an abundance of endospores. No spores were present in the interior of the cakes, but if the cakes were broken open spores developed on the broken surfaces in a few days, either in the ice chest or at room temperature.

At various times later, four more lots of Fleischmann's yeast (3 cakes in each case) were examined with similar results. Sometimes there were many spores present in the surface layers of the cakes as soon as they were received. Spores, however, always developed or became more abundant on keeping the cakes a few days.

The spores, which are quite refractive, may be observed fairly well by mounting them in a drop of Gram's iodine and examining under the microscope, using a magnification of about 600 diameters. Very satisfactory stained preparations may be made as follows: (1) spread a film in a small drop of water on a slide and dry in the air; (2) fix by passing through a flame 12 to 15 times; (3) stain with hot carbol-fuchsin 1-3 minutes; (4) wash with water; (5) destain with 5 per cent sulphuric acid 2-3 seconds; (6) wash with water; (7) stain with methylene blue about 3 seconds; (8) wash with water.

Cover and examine under the microscope. The spores should be stained a bright red and the vegetative cells or parts of cells a shade of blue. The wall stains only slightly, but may be seen satisfactorily if the mount is examined in water. If the cells are cleared in the usual way, and mounted in balsam, the wall of the ascus becomes invisible. The stained mounts are permanent if allowed to dry in the air, however, and may be examined at any time by covering with a drop of water and a cover glass. They may also be examined with an immersion objective

¹ TANNER, F. W., *The Yeasts*. Translation. 1920 (p. 152).

by applying immersion oil without a cover glass directly to the dry mount, but this renders the wall practically invisible.

Pure cultures may readily be obtained by the poured plate method, using potato dextrose agar (2 per cent dextrose) or a similar sugar medium. In such cultures some spores will develop in 4-6 days, especially on the upper parts of agar slopes. A much greater abundance of spores may be obtained, however, if transfers of the yeast are made from pure cultures to an agar medium prepared according to the following formula: distilled water, 100 cc.; Liebig's beef extract, 0.3 gm.; sodium chloride, 0.5 gm.; dextrose, 0.25 gm.; agar 1.5 to 2 gm. Sterilize at 12 or 15 pounds pressure.

On this medium,¹ which is a modification of that used by GORODKOWA, very good spore production resulted in 4-5 days, and in 8-12 days practically 50 per cent of all cells developed spores.

Cakes of "yeast foam" have also been examined, but no spores were found in them. When isolations from such cakes were made and the yeast grown on the medium given, spores developed apparently as readily as in cultures from a cake of Fleischmann's yeast.—W. E. MAN-EVAL, *University of Missouri, Columbia, Mo.*

¹ *Ibid.* p. 151.

CURRENT LITERATURE

NOTES FOR STUDENTS

So-called bars or rims of Sanio.—Since the publication in 1910 of Miss GERRY'S¹ stimulating paper upon their distribution in the Coniferae, the so-called rims or bars of Sanio have received considerable attention in discussions concerning the affinities of fossilized coniferous woods, and in controversies concerning the relative antiquity of the Abietae and Araucarieae. The conclusions of different investigators are extremely contradictory, not only as regards the nomenclature, form, and chemical composition of these structures, but particularly as regards their distribution in putative "conservative" regions or organs of the Coniferae.

In 1919 the writer² pointed out that unconformity between the primary and secondary membranes, and structures resembling the so-called bars or rims of Sanio, tend to appear at times in the Pteridophyta and Angiospermae, apparently as concomitants of processes of modification, especially reduction, in tracheary pitting. Although the writer suggested that the transverse thickenings in *Ginkgo*, *Pinus*, *Larix*, and other Coniferae may be vestiges of an ancestral scalariform structure, rather than modifications of curved rims or structures *sui generis*, he strongly emphasized the fact that until more is known concerning their developmental history, during differentiation from the meristems, the exact significance of the various types of primary pit areas and thickenings of the middle lamella must remain more or less obscure; therefore, their arrangement in phylogenetic sequences is purely conjectural.

SIFTON³ and HALE,⁴ in continuing the controversy concerning the relative antiquity of the Abietae and Araucarieae, focus their attention solely upon speculations concerning the significance of end products. The suggestion that transverse bars in Coniferae may be vestiges of a scalariform structure is divorced from its context in the writer's paper, and criticized without reference to the emphasis placed upon the necessity for detailed developmental investigations. SIFTON, in supporting THOMSON'S view of the primitiveness of the araucarians, cites the occurrence of bars of Sanio in the secondary wood and conservative regions of cycads as invalidating JEFFREY'S use of similar con-

¹ GERRY, ELOISE, The distribution of the "bars of Sanio" in the Coniferales. *Ann. Botany* 24:119-123. 1910.

² BAILEY, I. W., Structure, development, and distribution of so-called rims or bars of Sanio. *BOT. GAZ.* 67:449-468. 1919.

³ SIFTON, H. B., The bar of Sanio and primordial pit in gymnosperms. *Trans. Roy. Soc. Canada* 16:83-88. 1922.

⁴ HALE, J. D., The bars or rims of Sanio. *BOT. GAZ.* 76:241-256. 1923.

servative regions in araucarians. He maintains that bars are formed by vertical fusion between the rims of closely approximated primordial pits, in the higher gymnosperms by horizontal fusion of shorter bars and rims. Rims and primitive bars occur in conservative regions of *Pinus* and *Ginkgo*. On the contrary, HALE asserts that true bars or rims of Sanio characteristically occur in the secondary wood only of the Ginkgoales, Coniferales, Gnetales, and Angiospermae, and are absent in primary elements. The presence of bars in primitive regions of araucarians and their absence in similar regions of *Ginkgo* and *Pinus* is reiterated as evidence for the derivation of the araucarians from pinelike ancestors.

Although HALE errs in stating that unconformity does not occur in the tracheids of ferns and cycads, most discrepancies concerning the presence or absence of the so-called bars or rims of Sanio in different plants, organs, and tissues are due largely to varying definitions of these structures. Thus HALE coins his own definition of "true" bars or rims of Sanio, and brushes aside SIFTON's figures of bars in the secondary wood of *Dioon* as "not convincingly exemplified." It is significant in this connection, as previously noted by the writer, that the observations of KRÜGER, DEBARY, JANCZEWSKI, RUSSOW, STRASBURGER, DIPPEL, and others upon the occurrence of primary pit areas and thickenings in the walls of the cambium, and their relation to similar structures in the elements of the xylem and phloem, have been overlooked in discussions concerning the so-called rims or bars of Sanio. The writer's recent investigations upon the cambium and its derivative tissues strengthen his contention that extensive developmental investigations are essential if the protracted controversy concerning the significance of so-called bars or rims of Sanio is not to become a *reductio ad absurdum*.—I. W. BAILEY.

Respiration and photosynthesis.—In an important contribution, SPOEHR and MCGEE³ show that the carbohydrate content alone of leaves is no index of their respiratory activity. They show that the amino acid content of the leaves is very important. Using for most of the experiments excised leaves of *Helianthus annuus* and the "Canada Wonder" bean, they show that the general course of respiration in the case of leaves taken from the plant is downward. This is due to the decreasing carbohydrate content of leaves kept in the dark. If the leaves are fed sugar, this decrease in carbohydrates is much less pronounced. In this case respiration decreases during the first 30-35 hours, when there is an increase to a point higher than the initial rate. By numerous experiments, it is shown that this increase in respiration is due to an accumulation of amino acids in the leaves kept in the dark. The amino acids stimulate respiration, and, as a result, accompanying a decrease in carbohydrate content there is an increase in respiration. Such experiments, as well as experiments

³ SPOEHR, H. A., and MCGEE, J. M., Studies in plant respiration and photosynthesis. Carnegie Inst. Wash. Publ. 325. pp. iv+98. 1923.

showing a decrease in respiration correlated with an increase in carbohydrate content, show that the carbohydrate content alone cannot be used as a measure of respiration. When leaves were fed amino acids a stimulating effect on respiration was noted, especially when there was also sugar in the nutrient solution. Of the sugars used, d-glucose seemed to cause the greatest activity in respiration, d-levulose the least, while sucrose was midway between the two; d-mannose had much the same effect as d-glucose. Also, amino acids used in connection with d-levulose did not stimulate respiration, while the effect was marked in the case of d-glucose and d-mannose. The experiments give no explanation of this effect of amino acids, but, in the literature discussion, the effect of amino acids in stimulating enzyme activity is emphasized.

A few experiments are recorded showing a relationship between respiration and photosynthesis. If conditions were such that respiratory activity was low in the leaves, photosynthesis was also weak; and a high respiratory rate was associated with a high photosynthetic rate. SPOEHR prefers to let further experiments explain this relationship. In the introductory discussion some speculations are put forward, and the suggestion made that in this relationship we may possibly have an explanation of the internal factor of photosynthesis (WILLSTARTER's enzymatic reaction).

The literature citations are numerous, and the apparatus and methods are such as to insure the highest accuracy, and also to enable a large number of determinations to be made in a short time.—S. V. EATON.

Forest ecology.—The quantitative study of the factors of environment represents one of the most fruitful lines of investigation in modern ecology, and it is an indication of progress when foresters apply such methods to the solution of their problems. In a recent contribution, BATES⁶ tells of the collection of such quantitative data for climatic and soil conditions at the Fremont Experiment Station, Manitou, Colorado. These are to be summarized in a future publication, the present one dealing with the equally important physiological requirements of the principal tree members of the Colorado forests. The species receiving most attention are *Pinus ponderosa*, *Pseudotsuga Douglasii*, *P. Murrayana*, and *Picea Engelmannii*. These are compared as to their water requirement, sap density, non-available soil moisture, and resistance to high temperature and to winter drought. In the efficient use of both water and sunlight, as indicated by water requirement and sap density caused by abundance of carbohydrates, the spruce stands first, followed by the Douglas fir, lodgepole, and yellow pine in the order named. In the economy of water per unit leaf area exposed the order remains the same, except that the positions of yellow and lodgepole pine are reversed. This latter arrangement also expresses the resistance to winter killing. The economy in the use of both

⁶ BATES, C. G., Physiological requirements of Rocky Mountain trees. Jour. Agric. Res. 24:97-164. 1923.

water and sunlight energy on the part of the Engelmann spruce may explain to a considerable extent its shade tolerance and its success in competition.

Considerable attention is devoted to the relation of the trees to soil moisture, and it is interesting to note that the seedlings of the spruce, Douglas fir, and the yellow pine have essentially the same wilting coefficient, while the lodgepole pine has one somewhat in excess of the others. Here, as in the other studies, only seedlings are used, and BATES recognized that they may possess internal characters somewhat different from mature individuals, but, as he points out, it is the performance of seedlings rather than that of older trees which determines the composition of forest stands.

Too much emphasis can hardly be placed upon the importance of studies of this sort, especially when accompanied, as in this instance, with others of environmental factors. The task of correlating the two will not be a light one, but it promises to be most fruitful in leading to a much better understanding of the problems of forest development, whether under natural or under the partially controlled conditions of forestry.—GEO. D. FULLER.

A genetic study of *Splachnum*.—In addition to describing the morphology and anatomy of *Splachnum sphaericum* as it appears in nature and in cultures, SCHWEIZER⁷ reports the results of experiments with this moss suggested by the well known earlier work of É. and É. MARCHAL. The species proves to be strictly dioecious; that is, all the gametophores produced by a given protonema bear only antheridia or only archegonia. Secondary protonemata from any portion of a gametophyte produce gametophores of the same sex as that of the parental plant. From wounded portions of immature sporophytes protonemata were obtained which gave rise to (supposedly) diploid gametophores. Most of the diploid gametophytes, including protonemata and gametophores, closely resembled the haploid gametophytes; although numerous measurements showed differences in the average sizes of cells of certain types, the difference in each case being in favor of the diploid gametophyte. A considerable proportion of the diploid gametophytic clones were bisexual in the sense that each produced gametophores bearing antheridia and others bearing archegonia, but no bisexual gametophores appeared. Some diploid clones, however, so far as observation disclosed, produced only female gametophores. Three diploid clones constantly showed various marked aberrancies; two of these "monstrous" clones were strictly female, the third was strictly male. The eggs borne by the "normal" diploid gametophytes and by one of the "monstrous" forms were readily fertilized. This condition differs from that in the dioecious mosses investigated by the MARCHALS, the eggs of which proved incapable of fertilization. By the union of diploid antherozoids with diploid eggs, tetraploid sporophytes were obtained. Spores from these sporophytes gave rise to

⁷ SCHWEIZER, J., Polyploidie und Geschlechterverteilung bei *Splachnum sphaericum* (Linn. fil.) Swartz. Flora 116:1-72. 1923.

(presumably) diploid gametophytes, which to the time of writing had proved unisexual, four bearing only antheridia, three only archegonia. Wounded tetraploid sporophytes gave rise by means of protonemata to tetraploid gametophytes, which, so far as reported, had borne antheridia but no archegonia.—C. E. ALLEN.

Gametophyte and embryo of *Botrychium simplex*.—CAMPBELL⁸ has investigated *Botrychium simplex*, the smallest species of the genus, and related to *B. Lunaria*. The gametophyte closely resembles that of *B. Lunaria* in size and structure, but is much smaller than that of the other species examined. As in other species of the genus, the presence of a ventral canal cell is doubtful. More than one archegonium may be fertilized, but only one sporophyte was observed to develop from the same gametophyte. The embryo also resembles that of *B. Lunaria*, but the early divisions are variable and much more irregular. Details of embryo development are given and contrasted with other species. The vascular "skeleton" of the young sporophyte is said to be made up exclusively of the steles belonging to the roots and leaves.—J. M. C.

Protoplasmic streaming.—To make the demonstration of protoplasmic streaming easier, SEIFRIZ⁹ has studied the influence of various reagents on streaming in leaves of *Elodea*. He finds that methyl alcohol, the glucosides, saponin, senegin, smilacin, and strontium and barium chlorides, in dilute solutions of about two to five per cent, cause abnormal streaming after immersion for about two or three days. Copper in such concentrations as might occur in distilled water from a copper still also induces streaming. The types of streaming are described and suggestions as to possible causes of the stimulation are made.—W. B. DAVIS.

Embryogeny of Solanaceae.—SOUÈGES,¹⁰ in a series of four papers, presents the results of his investigations of the embryogeny of representative Solanaceae, the genera used being *Nicotiana*, *Hyoscyamus*, *Datura*, *Atropa*, and *Solanum*. A general summary organizes the situations that characterize the family.—J. M. C.

North American Flora.—The third part of volume 24 is a continuation of the Rosales by AXEL RYDBERG, including two tribes of the Fabaceae (Indigoferae and Galegeae in part). *Indigofera* is represented by 50 species, 11 of which are new. Of the tribe Galegeae six genera are presented, much the largest being *Cracca* (72 species, 16 new) and *Brongniartia* (37 species, 9 new).—J. M. C.

⁸ CAMPBELL, D. H., The gametophyte and embryo of *Botrychium simplex*. Ann. Botany 36:441-455. pl. 16. figs. 10. 1922.

⁹ SEIFRIZ, WM., A method for inducing protoplasmic streaming. New Phytol. 21:107-112. 1922.

¹⁰ SOUÈGES, R., Recherches sur l'embryogénie des Solanacées. Bull. Soc. Bot. France 69:163-178, 236-241, 352-365, 555-585. figs. 259. 1922.

THE BOTANICAL GAZETTE

October 1924

FACTORS CONTROLLING FOREST SUCCESSIONS AT LAKE ITASCA, MINNESOTA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 322

SHUN CHING LEE

(WITH EIGHTEEN FIGURES)

Introduction

From the standpoint of vegetation, Minnesota may be divided into two great regions by a line running from the northwestern to very nearly the southeastern corner of the state. Of these, the prairie occupies the southwestern and the forest the northeastern half. The forest region is divided into a coniferous area that may be regarded as a continuation of the northern coniferous forest formation, and a deciduous area which is an extension of the central hardwoods, with a steadily decreasing number of species toward the northwest (fig. 1).

Distribution of the three climax formations

CLIMATIC FACTORS.—It has been generally recognized that the main control of the distribution of the great climax formation of prairie, coniferous forest, and deciduous forest has been determined by climate. Low precipitation combined with high evaporating power is the limiting factor in the distribution of prairies. SCHIMPER (28) has shown that the prairie district of the United States is determined by the amount and seasonal distribution of the precipitation. The dry winter, the uniformly moist early summer, and the

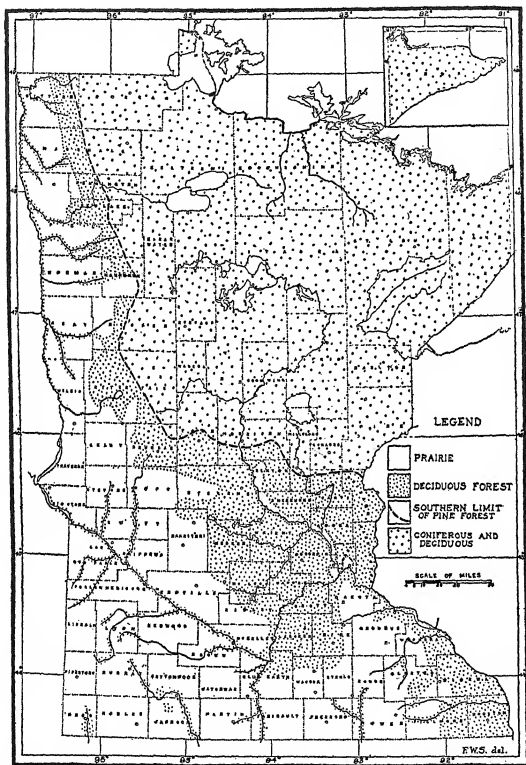


FIG. 1.—Map of Minnesota showing distribution of forest and prairie (after map by WARREN UPHAM and F. K. BUTTERS).

relatively scanty precipitation are typical of grassland climates. Thus evidently the distribution of prairie and forest in Minnesota is determined by the precipitation, not merely because of its small amount, but also by the character of its seasonal distribution. Fig. 2, after PURSELL (24), indicates a close correlation between the geographic distribution of the precipitation and the tension line of the prairie and forest.

Comparing figs. 1 and 2, one can readily see that the distribution of broad leaved and coniferous forests in Minnesota is not determined by precipitation, but by some other climatic factor or factors, among which undoubtedly temperature is the most important. The geographic distribution of any plant at its zero point depends upon its ability to reproduce itself. The survival and growth of seedlings and the consequent advance or retrogression of the particular forest type are determined by two main sets of factors: (1) environmental, principally temperature, which is responsible for the early and late killing frost and for determining the length of the growing season; and (2) the hereditary character of the different individuals, which gives special ability to withstand hard environmental conditions.

Most of the conifers, with thick cell sap and various xeromorphic structures, usually withstand a much lower temperature than do most of the broad leaved trees. This would naturally divide the forests of the temperate zones into two great belts: a cold temperate belt, with very cold winters, characterized by the conifers; and a warm temperate belt, with rather mild winters, characterized by the broad leaved trees. The border line between the two belts corresponds to the isothermal line of approximately 43° F. or 6° C. of the normal mean annual temperatures. This line is by no means sharp, however, a broad or narrow belt of transition always being found. The transitional belt between these two great types of forest in Minnesota runs along about the 45th parallel of north latitude, corresponding to the isothermal line of the mean annual temperature of about 42° F., and with the line of an average number of days of the crop growing season about 130 (fig. 3).

EDAPHIC FACTORS.—It is recognized that the distribution of the great plant communities is not governed by edaphic factors.

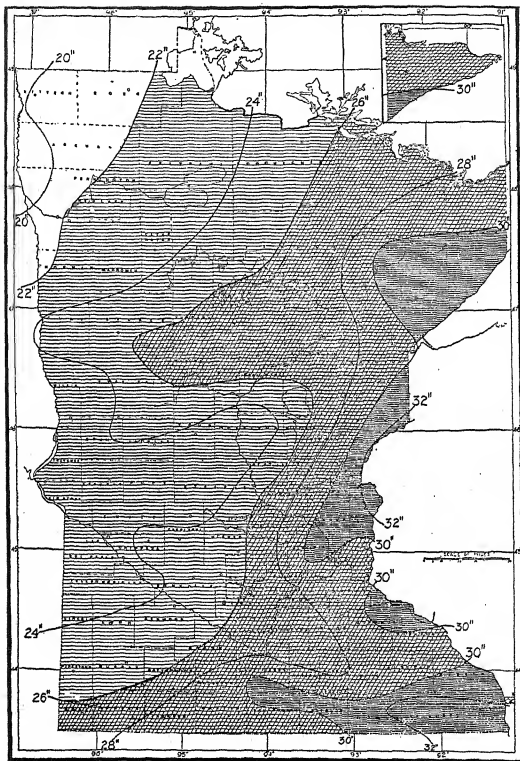


FIG. 2.—Map of Minnesota showing average annual precipitation (after map by U. G. PURSELL).

This general statement is especially well demonstrated by the distribution of soil and vegetational types in Minnesota. Broadly speaking, there are only three principal divisions of vegetation: west, prairie type; northeast, coniferous forest type; and southeast, hardwood forest type. The soil types, however, are extremely numerous in the different parts of the state. These soils are mostly glacial drifts, and consequently are extremely variable in nature and composition. The Minnesota Geological Survey gives nine principal surface soil formations (fig. 4), each of them containing a variety of soil types. Thus, if we compare fig. 1, showing the distribution of vegetation, with fig. 4, we find such a lack of agreement that we may safely conclude that edaphic factors do not determine the extent of the larger units of vegetation.

BIOTIC FACTORS.—Among the biotic factors are such plant and animal agencies as affect vegetation. The former comprise parasitic and saprophytic plants, as well as some of the lower forms of vegetation, such as algae, liverworts, mosses, etc. Their effect upon plant succession and the distribution of the larger plant communities is usually very slight. The latter group involves animals, insects, and human influences. The effect of this group on plant succession is usually very marked, and is seen in such changes as are due to grazing, insect invasions, cutting or burning forests, etc., all of which may occur in any place at any time, but are generally confined to rather limited areas. Thus, in the more important changes of a vegetation type these factors are of little significance, although repeated burning along the tension line between two types of vegetation will cause a continuous retrogression in the more advanced successional type. This condition is found in western Minnesota, where the retreat of the forest and the extension of the prairie type eastward are due probably to repeated injury of the forests by fires set by Indians to kill bugs or to convert forest lands into pastures. Although this may not change natural types in the main, constant destruction of forest cover will lower the water table, increase the evaporating power of the air, and bring about more xerophytic conditions, thus shifting the natural border line considerably. In this way it is evident that the distribution of the larger plant communities is controlled by climatic factors,

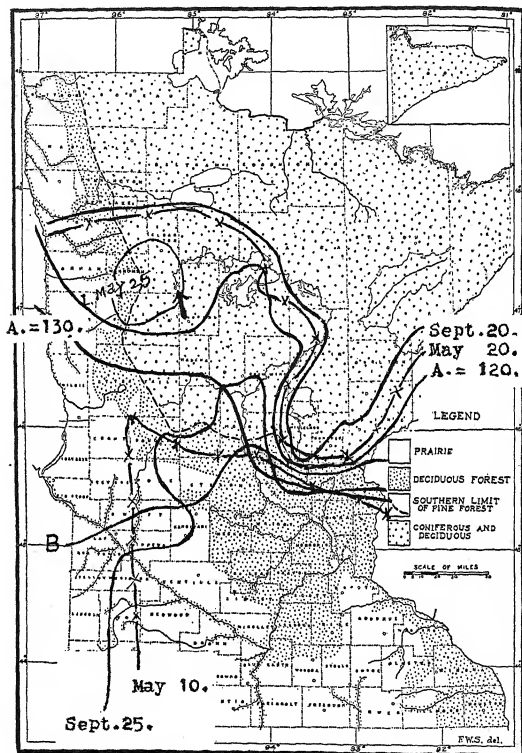


FIG. 3.—Map of Minnesota showing first and last killing frost date: *A*, number of days of average growing season; *B*, mean annual temperature of 42° F.; arrow pointing Lake Itasca.

among which the character and the amount of precipitation determine the extent of the prairie and forest type, and temperature determines the limits of the coniferous and hardwood forest formations. Both the conifers and hardwoods within the transitional belt are supposed to have an equal opportunity in distribution and development, for this belt contains the "zero point" of distribution for both types of forest. The purpose of this study, therefore, is an attempt to discover what factors play the most important rôle in forest successions within the transitional belt.

Study within transitional belt between coniferous and deciduous forest

LOCATION.—Lake Itasca is situated about 25 miles north of Park Rapids, in the southern tip of Clearwater County, Minnesota, 47° N, 95° W. The lake basin comprises an area of about 35 square miles. Lake Itasca is the headwaters of the Mississippi, and hence the basin has been made a state park. At the present time it is probably the only large tract of virgin timber left in Minnesota.

TOPOGRAPHY.—The region is a glacial drift plain dissected by water. The general topographic features therefore are a sandy plain with numerous morainic knolls and ridges dotted by more than a hundred lakes and swampy basins. The highest knoll is 200 feet above the lake surface, and the general relief is about 120 feet (fig. 5).

GEOLOGICAL AND SOIL CONDITIONS.—The entire region is covered by glacial drift. No rock outcrop occurs, but there are large boulders laid down by the ice. The soils are extremely variable, ranging from those composed of very stony and sandy material to heavy clay with few stones. The soil types are arranged in such a complicated manner that small areas of heavy clay soil are often found within sterile sandy plains, like the general character of glacial drifts in other regions. The glacial deposits also vary with the kind of country over which the ice passed. Thus, in the northeastern part of the state the drifts are composed mostly of volcanic and hard crystalline rocks of the region carried southward, while in the western and southern parts of the state the drifts are mostly of clay with imbedded limestone pebbles. All the calcareous

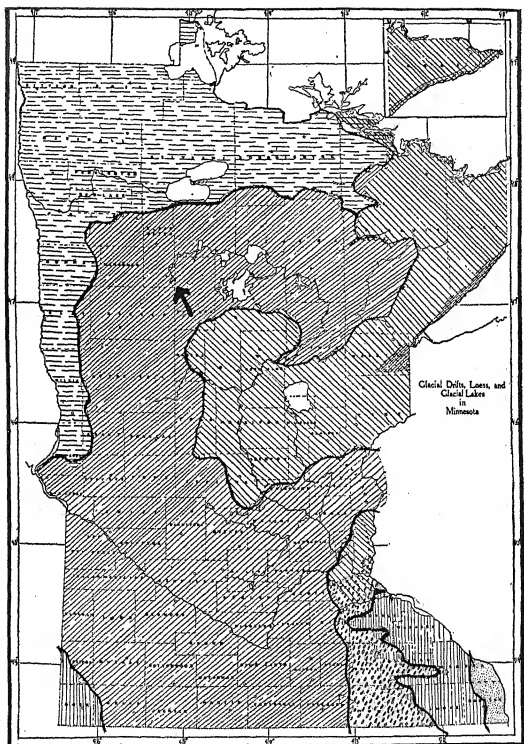


FIG. 4.—Map of Minnesota showing distribution of glacial drifts (after map by Minn. Geological Survey); arrow pointing Lake Itasca.

materials were gathered by the ice as it passed across the shale and limestone country of southern Minnesota, and these deposited in southwestern Minnesota are known as the gray drift. Being situated in a position to receive both types of the drift, Lake Itasca has an unusually large variety of soils.

In places where the soils are rather heavy and able to support a dense growth of vegetation, a layer of organic soil 5 or 6 inches thick has been formed. In places where outwashes occurred, the soil is sterile and unable to support a thrifty growth, and as a consequence very little organic soil has accumulated. Such areas are generally occupied by Jack pines or some other pioneer xerophilous vegetation. The swampy basins and lake shores receive yearly deposits of vegetable matter, hence the humus accumulation on these areas is very thick, and the soil is usually rich and fertile, but generally poorly drained; consequently such areas are usually occupied by black spruce, tamarack, or other xerophilous vegetation.

CLIMATE.—Minnesota has a mid-continental climate characterized by rather warm summers and cold winters. There is no prevailing wind and no high mountain range to chill the clouds and cause local convections, but a large proportion of the low pressure areas which move across the country from west to east touch the state. These areas are preceded by southerly winds and higher temperature and followed by northerly winds and low temperature, and are usually accompanied by cloudy weather and precipitation.

TEMPERATURE.—The average mean annual temperature shown by a continuous record of seven years is 38° F. The average temperature for January is 3° and for July 67° F. The highest temperature is 100° and the lowest—51° F. The average date of the last killing frost is May 25, and that of first killing frost September 15. The number of frostless growing days of the average crop growing season is 110.

PRECIPITATION.—The mean annual precipitation as shown by 25 years' records is 25.66 inches. Probably more than 50 per cent of the total annual rainfall occurs during the summer season, and therefore the winter season is usually dry. Of the mean annual snowfall of Lake Itasca there is no record, but that of Park Rapids is 46.2 inches, which would probably not be far from what we might

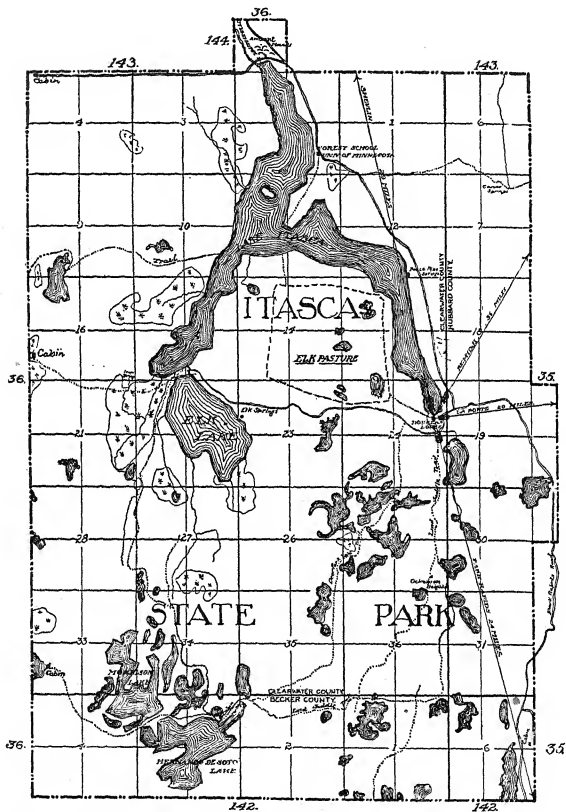


FIG. 5.—Map of Lake Itasca showing distribution of numerous lakes (after map by Minn. Forest Service Bull. 4).

expect at the Lake Itasca region. A rather detailed summation of the climatic components at Lake Itasca for the summer season of 1922 may help in understanding the climatic conditions during the growing season (table I).

TABLE I

MONTH	TOTAL RAINFALL (INCHES)	MAXIMUM TEMPERA- TURE	MINIMUM TEMPERA- TURE	NUMBER OF DAYS		
				Cloudy	Partly cloudy	Clear
June.....	3.19	94° F.	37° F.	21	6	3
July.....	2.56	92	41	16	8	7
August.....	3.10	101	36	21	5	5

FOREST CONDITIONS.—Probably two centuries ago the whole region was covered by white and red pine forests, as suggested by the remnants of dead stumps and logs. With the settlement of the region, the virgin forests began to be destroyed. This continued until 1891, when the region was made a state park, and some of the virgin pines have thus been preserved. In areas in which the virgin pines were destroyed either by fire or by the ax, a secondary growth of a mixture of jack and red pine together, with a number of deciduous species such as *Betula alba* var. *papyrifera*, *Populus tremuloides*, *Acer saccharum*, *Quercus rubra*, and *Q. macrocarpa*, were developed. The further development of these mixed stands depends upon the kind of soil; hardwoods usually occupy the heavier and more fertile soil, and the conifers the poorer sandy soils.

Among the virgin forests the following associations may be distinguished: (1) pure *Pinus resinosa* type; (2) pure *P. Banksiana* type, which abounds in sterile sandy areas; (3) *Acer-Pinus Strobus* type, which usually occupies fertile and heavy clay soil in limited areas; (4) *Abies-Picea* type, which usually occupies swampy basins or any depression where the moisture supply is abundant; and (5) *Larix* or *Thuja* type, usually found in the swamps or bogs.

Among the second growth five types may be recognized: (1) pure *Pinus Banksiana* type, mostly on sterile sandy soil; (2) pure *Populus tremuloides* type, mostly on very fertile soil; (3) pure *Betula alba*, usually succeeding the aspen stage; (4) hardwood

mixtures, composed of maples, oaks, aspens, birches, elms, and others, which generally occupy very rich soils; and (5) pine-hardwood mixture, composed chiefly of jack pine, red pine, aspen, and birch.

Beside these five types there is also a birch-fir-spruce type, which usually occupies lake shores or any depressions where soil moisture is abundant. On account of the numerous lakes, therefore, this type is predominating the region. In most places where the virgin red pines are standing, this type is developing as an understory. A cross-section of such a forest will show three distinct stories: the

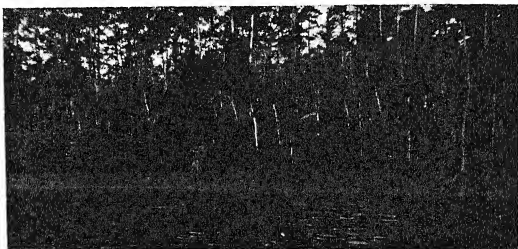


FIG. 6.—Virgin red pine forest at Lake Itasca showing three stories of forest: red pine, birch, and balsam fir-spruce, and showing invasion of balsam fir-spruce reproduction near lake shore.

old pines with open canopy first, the white birch second, and the thicket of balsam fir and spruce third. The age of the virgin trees is 150–200 years, and that of the second growths about 30 (fig. 6).

Forest successions

In studying the forest successions of any region, three periods must be considered, past, present, and future. The last one would naturally lead to a conclusion regarding the climax development of the region. Initial causes are usually classified into primary and secondary series, the former designating that succession which begins from denudation of either bare rock surface, sterile soil surface, or clear water edge, and the latter indicating that successions

may take place in any habitat where the existing vegetation has been disturbed by external factors such as burning, cutting, insect outbreaks, etc. For the natural classification of primary successions COOPER (9) introduced the terms xerarch (for land or xerophytic habitats) and hydrarch (for water or hydrophytic habitats).

PRIMARY SUCCESSIONS

HYDRARCH.—Hydrarch successions in northern Minnesota have been admirably studied by BERGMAN and STALLARD (3). The sequential development of hydrophytic successions from *Chara-Philotria* associates through *Castalia-Nymphaea*, *Scirpus-Zizania*, *Carex*, *Chamaedaphne-Andromeda*, as given by BERGMAN, leading to the *Larix-Picea* associates, is applicable to the situation of this region, but that through *Larix-Picea* and *Abies-Betula* leading to *Pinus* association, which BERGMAN called climax forest, seems hardly to apply to the conditions of the Lake Itasca region. The keynote of a climax forest consists not in the present predominance of a certain species, but in the spontaneous maintenance of its composition throughout future generations. Pines in this region are not only unable to invade *Abies-Betula* associates and reproduce themselves, but are being invaded by the latter (fig. 6). The intolerance of shade by pines compared with *Abies* and the thicket growth of the latter make it impossible for pines to culminate the successions in this particular region.

XERARCH.—The legend of fig. 7 gives a classification of stages of xerarch succession.

Past successions.—The denudation stage.—During the glacial periods this region was covered with ice, probably thousands of feet deep. The pre-glacial flora, therefore, must either have been killed off or swept away by the ice. After the retreat of the ice the region was covered with glacial drifts, upon which the plants started a new succession.

Herbaceous stage.—The initial substratum in this region, unlike many others, was bare soil instead of hard rock surface, and as a consequence the first stage of vegetation would be grasses and herbaceous forms. Among these, bunch grass, sedges, golden rods, etc., may have been present.

Shrub stage.—The availability of soil for a foothold for vegetation would shorten very decidedly the earlier stages of succession. Soon after the development of herbaceous forms xerophytic shrubs such as willows, birches, hazels, and other northern shrubs left behind by glaciers would have come in and driven out the herbaceous forms.

Pioneer forest tree stage.—By the gradually increasing density of shrubby growth the soil surface was shaded and the evaporating power of the air reduced. This resulted in a condition favorable for the germination of some of the pioneer forest tree species, notably *Populus tremuloides*, *Betula alba* var. *papyrifera*, *Pinus Banksiana*, and others that would constitute the first members of the forest succession.

Mixed conifer-hardwood stage.—After the establishment of forest trees the successional development in the region was controlled largely by the kind of soil and the tolerance to shade of different species. At this stage two principal types of soil may be recognized: (1) the sterile sandy areas resulting from glacial outwashes, which were occupied mostly by *Pinus Banksiana*; and (2) sandy loam and clay soils, which are fertile enough to support a dense tree population, upon which a mixture of hardwoods and the three native pines would have been developed.

Segregation of *Pinus resinosa* and the hardwood—*Pinus Strobus* stage.—Owing to the coarseness and looseness in texture and structure of the sterile sandy soils, the organic materials deposited on their surface and the mineral elements set free by physical and chemical agencies in the soil were constantly being leached out by rain water, and the improvement of soil conditions was very slow, and *Pinus Banksiana* continued to occupy this sterile habitat. Meanwhile the struggle for existence among the mixtures on the heavier soils was very keen. The degree of sensitiveness to soil moisture and to the supply of mineral salts and the ability to tolerate shade should mark a step in the succession. Consequently at this stage two types of forests occupying two types of soil would naturally have been segregated: clay soils with higher moisture holding capacity and with a higher percentage of nutritive salts, dominated by the more shade tolerant species such as *Pinus Strobus*, *Acer*

saccharum, *Tilia americana*, *Quercus rubra*, etc.; and the sandy and sandy loam soils with lower moisture and salt content, occupied by less shade tolerant and less moisture and nutrition exacting species, notably *Pinus resinosa*, the timber crop tree of the region.

Present successions.—The principal forest formations of the region at the present time are *Pinus Banksiana* type, *P. resinosa* type, and hardwood-*Pinus Strobus* type.

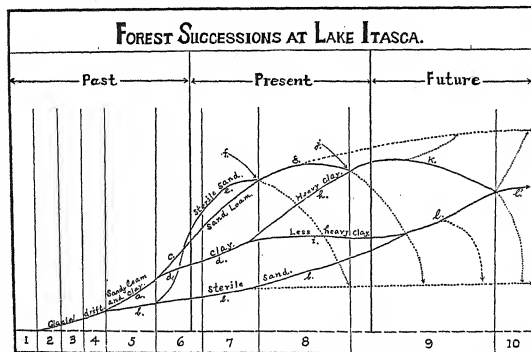


FIG. 7.—Advance of xerarch succession, entire lines indicating courses of primary and dotted lines those of secondary succession: (1) denudation stage; (2) herbaceous stage; (3) shrub stage; (4) pioneer forest tree stage; (5) forest stage (a, mixed conifer-hardwood type, b, pure jack pine type); (6) segregation of red pine and hardwood-white pine stage (c, pure red pine type, d, hardwood-white pine type); (7) edaphic climax and transformational stage (e, edaphic climax of jack pine type, f, transformational mixed jack and red pine type); (8) edaphic climax and parallel development stage (g, edaphic climax of red pine type, h, parallel development of hardwood-white pine type, i, invasion and development of *Abies-Picea* type, j, transformational and mixed red pine and hardwood-white pine type); (9) edaphic climax and parallel development stage (k, edaphic climax of hardwood-white pine type, l, parallel development of *Abies-Picea* type); (10) regional climax stage (m, *Abies-Picea canadensis* climax type).

Edaphic climax of *Pinus Banksiana*.—On account of the slow improvement of soil conditions in the sterile sandy soils, *Pinus Banksiana* is free from competition, and hence able permanently to occupy the areas where it became originally established.

Development to *Pinus resinosa* type.—Although most of the sterile sandy soils make imperceptibly slow improvement, some areas, especially those upon which *Pinus Banksiana* had reached its best development, would have been increasing in their organic matter content, and this would result in a decided increase in the moisture holding capacity of the soil, more of the rainfall would be made available, and less leaching would take place. The resultant improvement of the soil would make an invasion by *P. resinosa* both possible and probable. Such an invasion would result at



FIG. 8.—Dying off of *Pinus Banksiana* and transformational stage from *P. Banksiana* to *P. resinosa*.

first in a mixed stand of *P. Banksiana* and *P. resinosa*, and when two species of unequal ecological rank occur together, the inferior or more xerophytic usually gives place to the superior. *P. Banksiana* has a shorter life cycle and is more intolerant to shade than *P. resinosa*, and as a consequence the former is gradually dying out and being replaced by the latter (fig. 8).

Edaphic climax of *Pinus resinosa* stage.—A large percentage of the soils of this region being rather fertile sand and sandy loam, well suited to *P. resinosa* and a gradual increase in the areas of improved sterile sandy soils, conditions are favorable for the regional dominance of *P. resinosa*. This situation is probably present in other

parts of northern Minnesota, and has led BERGMAN and STALLARD (3) to conclude that the climax formation is one of pines. Among the pines they have included *P. resinosa* and *P. Strobus* growing in mixture, and they have further stated that probably the latter would finally replace the former. Conditions of this particular region, however, appeared to the writer such that neither of these two pines would be able to culminate the succession, simply because a true climax forest is not determined by the present quantitative predominance of a certain species, but by the continuity of their reproduction in the habitat which they are occupying. As a matter of fact, both *P. Strobus* and *P. resinosa* not only are failing to reproduce themselves, but are being replaced by either *Acer saccharum*, *Abies balsamea*, or *Picea canadensis* (fig. 6). The present predominance of *P. resinosa* is due simply to the amelioration and the suitability of soil conditions of the present stage, consequently the writer would call it an edaphic climax stage of *Pinus resinosa*.

Simultaneous development of hardwood-*Pinus Strobus* type.—On the limited areas of clay soil at this stage there is a simultaneous development of the hardwood-*P. Strobus* type. Among the hardwoods, *Acer saccharum*, *Tilia americana*, *Ulmus americana*, *Quercus rubra*, *Q. macrocarpa*, *Betula alba* var. *papyrifera*, etc., are the principal tree species. Since these different individuals are growing in a mixture, the competition between them is inevitable. The result of the struggle would again result in the dominance of the superior individual species, the inferior ones remaining in the less suitable habitats. *Acer saccharum* and *Pinus Strobus*, being more tolerant to shade and more exacting in soil fertility, would naturally occupy the heavy clay soils. Owing to greater tolerance of shade and more prolific reproduction, *Acer saccharum* may be able eventually to possess this habitat very completely, forming its climax. *P. Strobus*, on account of its weakness in reproducing itself under too heavy shade, may just fill up openings in the stand. It also seems that the somewhat lighter soils occupied by the other species of hardwood will be occupied, at some future time, by either *Acer saccharum*, *Pinus Strobus*, *Picea canadensis*, or *Abies balsamea*.

Invasion of *Abies-Picea*.—*Abies balsamea* and *Picea canadensis* constitute a large percentage of the forests in Minnesota, being

especially abundant in the northern part of the state, where there are many glacial lakes. In this region, however, no extensive areas of matured forest of these two species are found, but young stands of the two occupy probably more of the surface than do the seedlings of any other tree species, except the pioneer ones such as jack pine, aspen, birch, etc. These latter, as a rule, are abundant everywhere within their ranges. The most striking feature of these two species is that they reproduce themselves readily in any habitat that has room and an adequate moisture supply. Thus the less

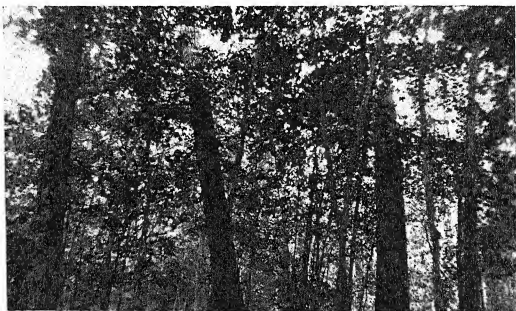


FIG. 9.—Mixed type of *Pinus resinosa* and hardwood-*Pinus Strobus*, with prolific reproduction of *Acer saccharum*.

heavy clay habitat occupied by hardwoods and having more open spaces and higher moisture content are more subject to invasion by the *Abies* and *Picea*.

Transformation to *Acer-Pinus Strobus* type.—It has been stated previously that *P. resinosa* cannot culminate the forest succession of this region. The northern limit of *Acer saccharum* extends as far as the Lake of the Woods, more than 50 miles north of this region. Although there are no large areas of matured maple forest, the prolific reproduction of this noted mesophytic climax tree appearing here and there (fig. 9) throughout the region indicates its future development. COOPER (9), too, called attention to the fact that

A. saccharum is dominant upon the main ridge at the southern end of Isle Royale, and that southward it is probably able to supersede the conifers and birch. All these indications make it hard to overlook this species as one of the most important competitors for the supremacy in forest succession of this region. Owing to the limited area of suitable soils the present development is slow, however, but the rapid improvement of the sandy loam soils occupied by *P. resinosa*, and the inability of pine to produce itself in its own shade, enable *A. saccharum* to develop fairly rapidly. Fig. 9 indicates the beginning of this transformation. *P. Strobus*, owing to its poor reproduction under dense shade, may hang on with the maple and fill up the openings of the stand, but cannot develop a large pure population.

Future successions.—Edaphic climax of *Acer-Pinus Strobus* stage.—Forests are very susceptible to damage by fire, animals, insects, wind, etc. No matter how well the region is being protected, these accidental injuries can hardly be avoided. Should there be no external disturbance, the constant improvement of soil conditions in the region would enable the *Acer-Pinus Strobus* type to supersede the *P. resinosa* and develop a climax. This climax stage being dependent upon soil conditions, the writer would call it an edaphic climax stage of *Acer-Pinus Strobus*.

Simultaneous development of *Abies-Picea* type.—As stated before, *Abies* and *Picea* reproduce themselves in any habitat that has space and adequate moisture supply. KENERY (16), through his intensive studies of *Picea canadensis* in northern Minnesota, concluded that the determining factor for its development is the soil, which has a direct bearing on the moisture supply. This statement shows a marked correspondence with the situation found in this region. Thus a parallel development of *Acer-Pinus Strobus* and *Abies-Picea* type in this region may be expected in future successions.

Abies-Picea climax stage.—Both the development of the mesophytic *Acer-Pinus Strobus* on its optimum soil condition, and the rapid development of the shade enduring *Abies-Picea* type, would lead one to conclude that a parallel development of these two types may culminate the succession, because so far as the tolerance to shade is concerned one type is just as shade enduring as the other,

and soil conditions are probably equally favorable and available for both types. Evidences, however, from quadrat studies on *Abies-Picea* reproduction (fig. 10), indicate that the struggle for supremacy

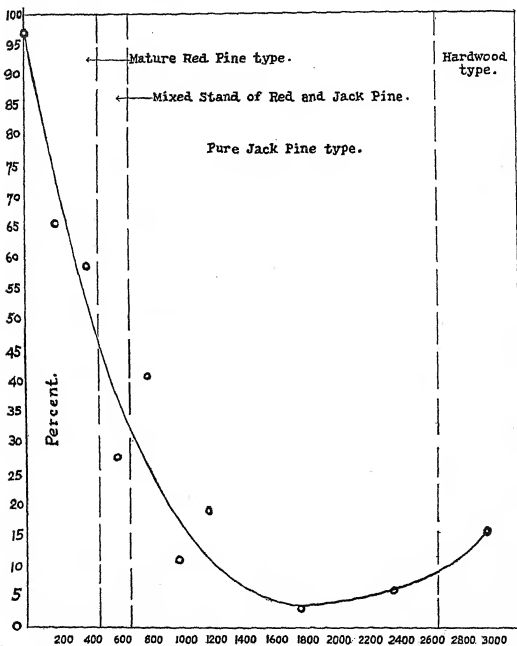


FIG. 10.—Percentage of *Abies-Picea* reproduction from lakeshore inland

in forest succession cannot end in the survival of both of these two types. This parallel development may last for a long time, but eventually the *Abies-Picea* type would probably dominate the climax of the region.

Quadrat studies in *Abies-Picea* reproduction.—The reproduction of the *Abies-Picea* association seems to be best near the margin of the numerous lakes, and less abundant in areas remote from such bodies of water. It seemed, therefore, that the nearness to such water supply as was represented by the lake water determined the relative richness of the reproduction. To test this assumption certain quantitative studies were made. Two parallel straight lines were run by compass at approximately right angles to the western shore of the big lake, for a distance of about one-half a mile. The lines passed through virgin stands of each of the four types of forest characteristic of the region (*Pinus resinosa*, mixed stand of *P. resinosa* and *P. Banksiana*, pure *P. Banksiana*, and the hardwood type). Along these two lines ten quadrats of one-fourth of an acre each (104 feet on each side) on each line were laid out with strings. The intervals between quadrats varied from over 200 feet to over 600 feet, depending upon the distribution of each type of forest crossed. Within the quadrats all the tree seedlings below 15 feet in height were tallied. Taking the total reproduction in each quadrat as 100 per cent, the percentage of *Abies-Picea* reproduction was calculated. The average percentage of the two lines or of the twenty quadrats was plotted as coordinates against the distance in feet away from the lake shore as the abscissae, and an average curve was drawn through the points (fig. 10). This curve shows that the percentage of reproduction of *Abies-Picea* increases in almost inverse proportion to the distance from the lake shore. This indicates that moisture is the determining factor for the development of the *Abies-Picea* type, and the effect of soil is only an indirect one, because even in the sterile sand or jack pine soils, when sufficient available moisture is supplied by the small lakes or depressions nearby, the *Abies-Picea* reproduction is found to be abundant. Since the region is thickly spotted with lakes and depressions (fig. 5), the writer ventures to predict that *Abies-Picea* will probably be able finally to supersede the hardwood-*Pinus* type. A further confirmation of this point may be found in the thicket growing habit of the former, which crowds out the latter very readily. Also other conditions being equal, the lower temperature and the shorter growing season in this region would naturally

favor the development of the northern *Abies-Picea* rather than that of the more southern species.

Secondary successions.—These begin after the destruction of the primary vegetation by external causes, chiefly lumbering and burning. After the removal of the trees, the forest floor is exposed to light. Then the evaporating power of the air is increased, the water table is lowered, and the nutrient constituents may be lost by washing. All these conditions tend to bring about a more xerophytic situation and cause retrogression in forest succession as a consequence. Retrogression, however, depends also upon the degree of destruction and the stage at which the destruction occurred; if a forest is cut with such care as not to cause deterioration of the soil conditions, and at such a stage of a good seedling year as would assure an immediate reproduction of the same species in the habitat, there would be no retrogression in the succession; on the other hand, a reckless cutting may set back the succession for two or three stages.

Burning, unlike the cutting, removes not only the forest stand but all other vegetation as well. As a consequence, in addition to bad conditions brought about by destruction, organic materials are burned up and nitrogen in the soil is liberated, and thus plant succession may be set back to the primary denudation stage. On the other hand, if the fire is not too severe, the burning may be beneficial to forest succession, as light fires usually help to kill back the underbrush, open resinous cones, stimulate germination, and encourage the development of the major forest tree species. The best illustration of such beneficial burning is probably seen in the Douglas fir forest in the Pacific northwest. HOFMANN (14) says, "the principal combination of factors responsible for the preponderance of the Douglas fir formation is the presence of seeds stored in the forest floor, and of fire." When the forest is removed by fire, the stored seeds get conditions favorable for germination and immediately respond to the stimuli. He further concludes that "the Douglas fir formation (*Pseudotsuga-hyllium*) is not, as might appear, a retrogression from the cedar-hemlock climax, but is rather a stage of succession preceding the climax."

In general, destructive lumbering and severe fires almost always cause retrogression in forest succession. The dotted lines of fig. 7 indicate where the retrogression of different stages of succession on various habitats may fall. The development of the secondary succession, like that of the primary, depends upon the ability of aggregation, migration, establishment, and competition of the individual species and the differences in initial stages at which the secondary succession commenced. The vegetational cycle of primary successions will then be followed and repeated.

Controlling factors in forest succession

It is obvious that forest successions are controlled, not by a single factor or by a few factors, but by a complex of factors, hence a detailed treatment of all the possible factors, together with some instrumental evidence secured from this region, should be presented. Among the complex of factors, three main divisions are commonly recognized.

CLIMATIC FACTORS

The climatic set comprises all such atmospheric components as humidity, precipitation, temperature, which may be termed direct factors. The evaporating power of the air, which is a summation of all the climatic components which influence the atmospheric control of the water supply, may be termed an indirect factor. The direct climatic factors usually influence the forest successions uniformly over a widespread region. The plant succession that takes place in response to the widespread climatic influence, COWLES (10) has defined as regional succession. This involves long periods of time, and through the operation of climatic agencies may be expressed in terms of eons. Because of the widespread uniformity of climatic factors and the relative permanency of their duration, it seems, according to NICHOLS (21), that much more is to be gained than lost by ignoring some of the climatic agencies and directing attention to the nearer physiographic and biotic factors, whose operation may occur within periods of centuries and decades respectively (10). NICHOLS (22) also points out that "in applying the law of the minimum to problems in local physiographic ecology,

climatic factors need not be taken into account, since they are essentially constant throughout the region."

EVAPORATING POWER OF AIR.—Owing to the great variation in vegetation and the lack of adequate instruments devised for measuring even the approximate amount of water lost by transpiration, and lack of means of measuring the amount of water lost through runoff and by percolation, it seems impossible at present to attack the problem of plant growth in relation to the amount of the precipitation of the region. LIVINGSTON (19) tried to solve this difficult problem by devising the atmometer cups to measure the evaporating power of the air, which is the summation of all the atmospheric factors that determine and limit the water loss by plants. The researches of LIVINGSTON (19), FULLER (13), THONE (29), and others have shown some satisfactory results from the use of the atmometer. According to FULLER, the differences in evaporating power of the air alone would be sufficient to account for certain advances in successions. Thus the evaporating power of the air in different types of forest would help to explain the forest successions in this region.

Livingston spherical atmometer cups equipped with the rain-proofing valves as devised by LIVINGSTON and THONE (20) were employed. The reservoir bottles were sunken partly into the ground, so that the cups were about 8 inches above the surface, with one exception, which was hung on a drooping branch about 6 feet from the ground, for measuring the conditions above the undergrowth. Eight stations were established in seven different forest types scattered over the region. Six stations were visited once every five days, and two irregularly. All the readings, except those of the two stations that were taken irregularly, after correction, were plotted (fig. 11). The average daily evaporation rates of the different types for the whole period was reduced, and these, together with mean daily precipitation in cubic centimeters of the period, are shown in fig. 12.

Figs. 11 and 12 show a decided contrast in the evaporating power of the air in different types of forest. The open field, owing to free air movement, higher wind velocity, and stronger insolation, naturally has the highest rate. The cutover land, with scattered

shrubs and primary forest trees, and a resultant checking of air movement and wind velocity, has an evaporation rate second to the

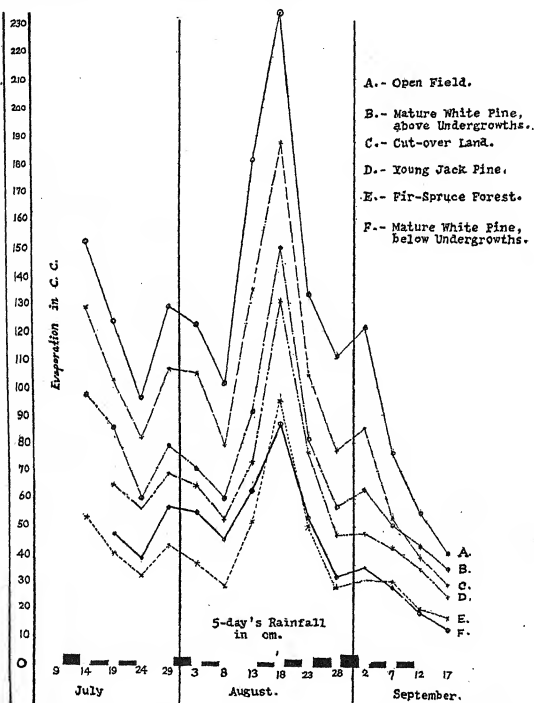


FIG. 11.—Evaporating power of air in different types of forest in 5-day period, and amount of rainfall of equivalent length of time.

open field. In the matured red pine forest, owing to open canopy and lighter soils supporting only a limited undergrowth, the rate is

ranked fourth. The records of the matured jack pine type, owing to disturbances by animals during the course of the investigation, cannot be presented. Judging from a few days' complete record, however, it would be ranked third in the series. In the young jack pine type, owing to its thicket habit of growth, the rate of evaporation is ranked fifth. According to this investigation, the rate of evaporation has probably less influence on succession during juvenile stages. In the matured white pine type, on account of very

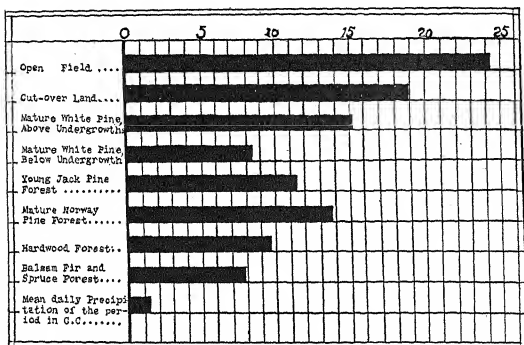


FIG. 12.—Average daily rate of evaporating power of air of different types of forest

dense undergrowth, two stations (one below and the other above the undergrowth) were established. The rate of evaporation at the station above the undergrowth is ranked third, and that of the station below the undergrowth sixth. The hardwood type, with high mesophytic conditions, is ranked seventh, and the *Abies-Picea* type, with its dense shade, is seen to be the most mesophytic of all the types.

These instrumental data show a marked correspondence with and give a greater emphasis to the sequence of forest succession as previously given. Thus, assuming that all the other factors are equal, the differences in the rate of evaporation would tend to cause a sequence

of succession as outlined. Nevertheless, the records of the evaporating power of the air in the lowest stratum of the various forest types for one season is far from sufficient evidence upon which to base any conclusion. The data of FULLER (13) and WEAVER (31) from records extending over three consecutive years, however, prove that while the records in a series of associations varied somewhat from year to year, the same general proportionate relationship was maintained between various associations, and the sequence in mesophytism between the various forest types remained the same throughout the entire period. This unchanging order between the different types in their rate of evaporation is sufficient to justify the belief that one season's records is substantially indicative of the permanent relationship of the communities, and that records extending over many years would result in no essential differences.

EDAPHIC FACTORS

This set of factors includes all those conditions of the substratum that either directly or indirectly influence the growth and development of vegetation. The former comprises soil composition and soil nutrients, soil acidity and alkalinity, soil temperature, soil moisture content, depth and texture of soil. The latter involves surface cover and topography and slope.

DIRECT FACTORS.—*Soil composition and nutrients.*—Broadly speaking, the soil is composed of two general classes of material, minerals and organic matter. The former may be subdivided into two groups; mineralogical constituents (chiefly quartz, which makes up the bulk of the soil), and the nutrient productive particles (such as compounds of aluminum, calcium, magnesium, potassium, sodium, sulphur, iron). All these three kinds of soil particles are found in practically every kind of soil. The solubility and presence or absence of certain mineral elements determine the chemical properties of the soil, and that of the organic materials has more to do with the physical and biological phases. Their relative proportion and availability determine what is generally called the soil fertility. In speaking of soil fertility all three phases must be taken into consideration. The soils of this region have not been studied intensively by any soil specialist. LEVERETT (18) gives only general

information about the surface formation, and ALWAY (1) gives a good account of Minnesota glacial soil studies, but no detailed information for this particular region. The soils are here so intricate as to require a detailed study; therefore no attempt at chemical analysis will be made in this paper, but some of the peculiar characters of the soils that usually support special types of vegetation may be mentioned. The most striking characters are those due to the presence of rich iron compounds and calcareous elements. The former are especially abundant in coarse sterile sand, and more prominent at a depth of 2 feet or more. This may be due to the fact that at greater depths the soil becomes coarse, and the coarser particles are usually chemically inert; consequently more insoluble iron compounds are deposited. RUSSELL (26) calls attention to the fact that soils containing ferrous compounds are generally infertile. This may explain the lack of fertility in the reddish sterile sand here occupied by jack pine. The calcareous materials, on the other hand, decidedly influence soil conditions. Directly they furnish plant food, and indirectly they change the physical condition so as to increase the soil tenacity and soil moisture-holding capacity. The latter is especially important in this region of moderately low precipitation, and has a direct bearing on forest succession. Thus the relative proportion of calcareous materials seems to determine the forest types; the sandy loam soils with less calcareous material being generally occupied by red pine, and the clay soils with a higher calcium content by hardwoods and white pine type. FERNOW (12) and HOWE (15) call attention to the fact that the composition and distribution of forests vary mainly according to the differences in the soils. Fig. 13 shows the sensitiveness of forest types in response to soil conditions. The boundary line of the two forest types agrees so closely with that of the two soil types that the former look like an artificial plantation. The texture, structure, and composition of the soil thus play an important rôle in the forest succession in this region.

Soil acidity.—It is a widely accepted fact that different kinds of vegetation have differences of acid tolerance. The correlation between the hydrogen-ion concentration of the soil and the degree of acid tolerance of the plants determines to a considerable extent

the distribution of the vegetation, and consequently modifies the plant succession. Certain plants, such as species of *Sphagnum*, *Osmunda*, and *Vaccinium*, are usually referred to acid soils, and others, like *Amaranthus*, *Tamarix*, *Chenopodium*, and *Atriplex*, are usually found in more or less alkaline soils. On account of the supposed limitation of such plants to acid or alkaline soils, ecologists have often taken them as indicators of soil conditions. This concept of soil indicators must not be too rigidly held, because more recent investigations show that inferences drawn from the

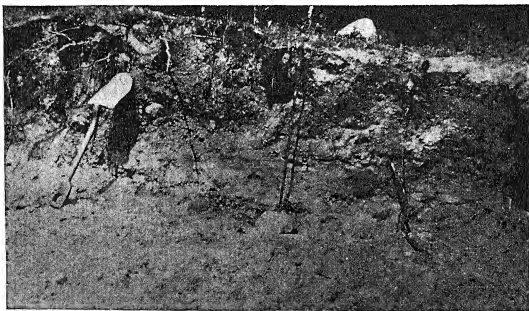


FIG. 13.—Calcareous substratum underlying coarse sand soil supporting *Pinus resinosa* type on left, and same soil without calcareous substratum supporting *P. Banksiana* type on right.

presence of such supposed indicators are not always correct. *Pinus Banksiana*, *Arctostaphylos Uva-ursi*, *Gaultheria procumbens*, and *Maianthemum canadense* are commonly recognized as acid indicators, but the recent work of KURZ (17) shows that they may be found upon soils with an alkalinity as high as that indicated by P_H 7.5. Thus it is not safe to adhere too rigidly to the idea of acid and alkali plant indicators without taking account of such factors as aeration, soil moisture, and light. KURZ also points out that one must distinguish acid clay, acid sands, and acid peats, which evidently means that the P_H value probably does not account for the

distributional phenomena to such an extent as does the difference in soil types. This statement agrees with the condition of the plant association in this region. In the sterile sandy soils Jack pine is invariably associated with *Arctostaphylos Uva-ursi*, *Vaccinium canadense*, *V. pennsylvanicum*, and *Maianthemum canadense*, while in the loamy and clayey soils occupied by red pine and hardwoods

TABLE II

Soil type	Depth	No. of samples	P _H
Sterile sandy soil....	Jack pine		
	6 inches	3	5.5
	6 inches	5	6.0
	6 inches	2	7.0
	1 foot	10	6.0
	1 foot	8	6.5
	2 feet	3	6.0
	2 feet	3	5.5
	3 feet	4	6.0
	Red pine		
	6 inches	2	7.0
	6 inches	6	6.0
Loamy soil.....	1 foot	12	6.0
	2 feet	4	6.5
	3 feet	4	6.0
	3 feet	1	5.5
	Hardwoods		
	6 inches	7	6.5
Clayey soil.....	6 inches	5	6.0
	6 inches	1	7.5
	1 foot	6	6.0
	1 foot	2	6.5
	2 feet	3	6.0
	2 feet	2	6.5
	3 feet	3	6.0
	3 feet	2	5.5
	3 feet	1	5.0

one can hardly find one of them. This situation would imply that the sterile jack pine soil must be more acid than the others, but the fact is not universally true. About forty soil samples taken from a depth of 6 inches in various soil types of the region were tested by KURZ, and about seventy samples taken from a depth of 1, 2, and 3 feet in various types were tested by the writer with the LaMotte chemical indicators. The results are given in table II.

Table II shows a remarkable uniformity of the P_H values throughout the region. Most of them range from P_H 6.0 to 6.5, with only two exceptions, P_H 5.0 and 7.5 respectively. Also the greater the depth from which the soil samples are taken the more uniform is the hydrogen-ion concentration. Because of these uniform conditions of acidity throughout the region, the writer is inclined to believe that soil acidity has probably no significance in the forest succession. The invariable association of *Pinus Banksiana*, *Vaccinium pennsylvanicum*, and *Arctostaphylos Uva-ursi* seems to the writer to be due not to the acid influence but to the sterile condition of the soil, in which the other plants are unable to grow. Thus, in any habitat that is sterile, competition between the plants is usually not keen, and only the hardy species will survive as a natural association, and they may mislead one into believing that they are acid soil indicators.

Soil temperature.—It is well known that soil temperature does not fluctuate much during the year, and a constant temperature is reached at a depth of 34 feet. The most prominent fluctuation occurs only within the upper 2 or 3 feet. In the forest, owing to dense shade, the fluctuation of soil temperature is much less than that in the open. Nevertheless, it is of great significance to both soil fertility and the development of plant roots; consequently it bears a definite relation to forest succession. WEAVER (31) in his work in Idaho and Washington found a marked relation between the temperature of the soil at various depths and the stage in the succession; he found that the temperature decreases as the climax community is approached. His work (32) at Lincoln, Nebraska, also shows that the soil temperature becomes progressively lower at all depths, from prairie through scrub to forest. The progressive decrease of soil temperature as the climax community approaches is probably due to at least two causes, the increase of shade and the consequent decreased amount of direct insolation, and the increase of soil moisture content as the climax community is approached and the consequent lowering of the soil temperature. The kind of soil should be taken into consideration, of course, sandy soil generally being more readily heated and more rapid in its loss by radiation; while clay both absorbs and loses its heat more slowly, and

has a very uniform temperature in consequence. Furthermore, soil receives heat, not merely from insolation and from the interior of the earth, but also from the decay of organic matter. Rich soils supporting a dense vegetation have more organic matter subject to decay, consequently more heat would be produced by such decomposition. Thus the lower temperature in clay due to higher moisture content may be compensated to a great extent by the products of decay. The soil in this region, as stated before, ranges from bare sand to heavy clay. The records of soil temperature, therefore, followed the natural sequence; that is, the open Jack pine forest with little or no humus cover had a higher average soil temperature than the mesophytic hardwood. It is regretted that, owing to lack of adequate instruments, the soil temperature of various types could not be measured for a considerable length of time; however, a two weeks' record of various types may give a general idea of the sequence (table III).

TABLE III
AVERAGE SOIL TEMPERATURE IN CENTIGRADE OF
SEPTEMBER 3-16, 1922 INCLUSIVE

Forest type	6 inches	1 foot	2 feet	3 feet
<i>Pinus Banksiana</i>	14.31	14.09	14.16	14.20
<i>Pinus resinosa</i>	14.28	14.10	14.14	14.16
<i>Acer saccharum</i>	14.10	14.01	14.08	14.09
<i>Abies-Picea</i>	13.82	13.78	13.80	13.81

Table III indicates the general sequence of the soil temperature and the different forest types. One should bear in mind, however, that, although the soil temperature is very significant to forest succession, it acts only as an indirect factor, because soil temperature is determined by the kind of soil and the type of forest rather than the reverse. The sterile sand soil, with little or no humus cover, will be frozen earlier in the winter, and, being deficient in shade, will be heated more quickly and dried out more easily in the summer; consequently it will tend to support only pioneer vegetation. On the other hand, the heavy clay soil with slower fluctuations would tend to support a more mesophytic forest.

Soil moisture.—The vital importance of soil moisture to the vegetation has been recognized from the very beginning of ecological

sciences. WARMING (30) classified the distribution of plant communities largely upon moisture conditions. SHREVE's work (27) shows that soil moisture is the factor that determines the occurrence and distribution of vegetation. PEARSON's (23) experiments showed that by supplying adequate soil moisture nearly all the species found in the western mountain region are capable of enduring high temperature, and of growing under conditions of lower atmospheric humidity. Thus it becomes evident that soil moisture in relation to plant growth requires critical consideration. Attempts have been made by many workers to establish a definite relation between the moisture supply and plant growth. BRIGGS and SHANTZ (4) first developed the idea of wilting coefficient. ALWAY (1) separated the water into available and non-available by means of the hygroscopic coefficient. CLEMENTS (7) classified soil water into chresard, involving the available, and echard, involving the non-available portion. Since it has been proved that the ability of one plant to secure soil water is little different from that of another, a single valued numerical term to express the soil moisture has been established by BRIGGS and McLANE (5), known as the "moisture equivalent." On account of adequateness this method was used to secure soil moisture data for studying the forest succession.

The records of all the previous investigations show that the moisture equivalent varies with the different kinds of soil and with the various depths. The general situation is that the moisture equivalent is usually higher on the surface and lower within the subsoil of the same habitat. The critical depth for the development of seedlings probably is not more than 1 foot; therefore, soil samples for this investigation were taken from various depths within a foot of the surface, and from various places within the region, so that a better average for each type of soil as well as each type of forest could be secured. The samples were tested by using a centrifugal force of 1000 times gravity. The average results and the range between the different types of soil and their occupants are given in table IV.

Table IV indicates that the range of moisture equivalent exhibits a distinct correspondence with the sequence of the forest succession, with one exception, the *Abies-Picea* type, which according to the successional series should occupy the soil with the highest

moisture equivalent. As stated before, however, the *Abies-Picea* type is found in any kind of soil that has space and adequate moisture supply. It should be clear, therefore, that a high moisture equivalent does not necessarily mean an adequate moisture supply, nor a low equivalent one that is deficient. For example, the sand has the lowest moisture equivalent of the series, but when it is near to some of the many small lakes or ponds the underground source of supply provides a constant and abundant moisture content. Remote from such open bodies of water, however, it is necessary that the soil possess a considerable water-holding capacity to provide a reserve for the vegetation during the critical dry period of the year.

TABLE IV

Forest type	Soil type	Average moisture equivalent of types
Young <i>Pinus Banksiana</i>	Coarse sand	1.2
Matured <i>Pinus Banksiana</i>	Medium sand	2.6
Matured <i>Pinus Banksiana</i>	Fine sand	8.6
<i>Abies-Picea-Betula</i>	Fine sand	9.1
<i>Pinus resinosa</i> - <i>P. Banksiana</i>	Sand loam	9.9
<i>Pinus resinosa</i>	Sand loam	10.7
<i>Pinus resinosa</i>	Loam	11.2
Mixed hardwood	Loam	14.2
Mixed <i>Pinus resinosa</i> and <i>P. Strobus</i>	Clay	18.3
<i>Acer-Pinus Strobus</i>	Heavy clay	22.4

Thus, aside from the proximity of open bodies of water, the difference in the soil type determining the amount of moisture supply together with the difference in the rate of the evaporating power of the air would determine the forest succession of the region.

Depth and stratification of soil.—In many regions, especially in hilly countries where the soil mantle is usually thin, the relative thickness of soil often becomes a limiting factor in plant succession. In clay country the impervious stratum known as the hardpan is generally present. The poor drainage and bad aeration of such a habitat determine a special type of vegetation. Soils in the region under consideration (except in the bogs and swamps) are remarkably deep, with excellent drainage and aeration, therefore this factor may be disregarded in the discussion of forest successions.

INDIRECT FACTORS.—*Surface cover.*—The surface cover may be divided into two groups, the living cover which includes all the

shrubs, herbs, and grasses, and the non-living cover which includes all organic debris and snow. These two groups have much indirect influence on forest successions.

Living cover.—In these forests nothing perhaps is more striking than the abundance of undergrowth, consisting mainly of *Corylus*



FIG. 14.—Adventitious roots of *Corylus rostrata* within area of 3 feet square

rostrata, *C. americana*, and *Aster macrophyllus*. The first two usually form a dense first story, and the last the second story of the undergrowth. The prolific propagation by means of both seeds and adventitious roots enables them to develop so abundantly that practically no other kind of vegetation is able to grow under them. The soil of the upper 6 inches is practically fully interwoven by their roots and underground stems (figs. 14-16). This thicket growth of underbrush has a tremendous influence on the existence

and development of forest tree seedlings. The competition for space, light, water, and plant food between the tree seedlings and the underbrush becomes very keen. The feeble pine seedlings cannot conquer the dense and thrifty undergrowth. The failure of reproduction in the pines is here largely due to the presence of

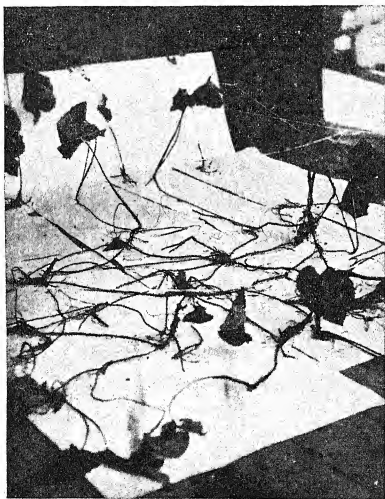


FIG. 15.—Adventitious roots of *Aster macrophyllum* within an area of 3 feet square

this thicket undergrowth. This fact is well indicated by the presence of many one-year old pine seedlings here and there under them, and the entire absence of such seedlings of any greater age. During the spring, which is usually rather dry, the soil moisture in the surface layer (which is generally of duff or humus and not very intimately connected with the subterranean capillary moisture supply) would easily be exhausted by this heavy foliated under-

growth. Then the dense shade combining with a critical desiccation would be sufficient to kill the feeble pine seedlings.

Non-living cover.—The amount and kind of debris have a decided influence on the radiation and absorption of the insolation, and as a consequence it affects the soil temperature. It also affects the water content of the soil by checking the run-off, increasing the absorption, and lowering the rate of surface evaporation. Here again, the jack pine soil with little or no humus cover has a wider range of soil temperature and a higher evaporation; consequently it is more xerophytic. On the contrary, the hardwood-*Pinus Strobus* forest soils have thick humus cover and are more mesophytic. The duration of snow in different types of forest has also a great influence on the soil temperature and soil moisture condition. In the dense shade of the coniferous forest the snow usually lasts longer, keeps the ground from being frozen, and reduces the loss of water by evaporation; while in open jack pine forest the snow melts more quickly, and the soil is more subject to freezing. All these influences have a share in determining forest succession, but they are of minor importance.



FIG. 16.—Mixed type of *Pinus resinosa* and *P. Strobus*, showing density of undergrowth.

Topography and slope.—Practically all the earlier work in the field of ecology recognized the general effects of topography as one of the most important modifiers of plant succession. COWLES (11) especially emphasizes the importance of topography as a general control over other factors in dynamic plant successions. The dynamic topography or the slopes that are subject to active erosion, strong wind action, and to unequal insolation afford efficient causes for plant succession. In a flat country, however, especially with sandy soil, as in this region, the topography changes slowly and has very little effect on the plant succession.

BIOTIC FACTORS

Among the biotic factors two main groups may be recognized: phytobiotic and zoobiotic. The former includes all influences of plants themselves, and the latter all those of animals and insects.

PHYTOBIOTIC FACTORS.—Tolerance and light intensity.—The ability of trees to endure shade is generally called tolerance by the forester. Since light is essential for photosynthesis and assimilation, the relative amount required by each species and the light intensity available become one of the most important factors in forest succession. The tolerance of shade varies not only with different species, but even within the same species, according to the conditions under which the tree is growing. WIESNER (33) states that trees increase their tolerance when they are growing in more fertile soils and under more suitable moisture conditions, and the same individuals would be less tolerant to shade when they grow in precarious conditions. Besides the soil and soil moisture factors, the age, vigor, origin of the tree, and all the climate factors have bearings upon its tolerance. Because so many different factors affect the tolerance of the tree it is extremely difficult to measure the tolerance of a given species, and an exact qualitative value of tolerance of a certain species of tree cannot be found. ZON (34) follows a number of European authorities in proposing a relative scale designating the tolerance of the different tree species. Thus, instead of measuring the amount of light a certain species requires, investigators generally measure the relative

amount of light intensity present in the different types of forest. To measure the light intensity WIESNER first devised an "insolator" and used a sensitive photographic paper. Following the same principle, CLEMENTS (7) invented a "photometer." Recent ecological investigators such as BROWN (6), THONE (29), and others have employed LIVINGSTON'S "radio atmometer" as a means of getting the light intensity through the difference in the evaporation from black and white cups. None of these instruments and means has proved satisfactory. In this study, for the sake of convenience, two thermometers, black and white, were employed. This method, of course, is by no means accurate, but it gives the relative value of the light intensity in different types of forests with a fair degree of accuracy.

The bulbs of ordinary thermometers were wrapped with silk cloth, one with black and the other with white. The two thermometers were then attached to a narrow grooved board. Since we know that black absorbs and white reflects, the difference in readings between the thermometers would be due to the light intensity. The thermometer board was held facing horizontally upward at about 4 feet from the ground, and was carried slowly through the forest along definite lines laid out in such a way that they would cover all the different conditions of the stand. Readings were taken at every 200 feet, and in order to afford sufficient time for the thermometers to adjust themselves to the different light conditions, 10 minutes were allowed for each reading. After each reading the thermometer board was put in a light-proof sack until both instruments indicated the same reading. They were then taken out and the next reading made after the 10 minutes interval. The readings were taken at the same time on the different days. The days for such investigations were of about the same approximate brightness, which was judged by exposing this thermometer board to direct sunlight at the same spot and the same time of the day. The readings thus indicate the difference in brightness. Fifty readings were made in each type of forest. The average results of the different types are shown graphically by fig. 17. The relative light intensity between the different types is expressed quantitatively in table V.

As can be seen from table V, by taking the matured jack pine type as a standard and its ratio as 1, the intensity in the hardwood type is $1/7$ and that of the *Abies-Picea* type only $1/10$. The weak light intensity in hardwood and *Abies-Picea* type apparently would eliminate the reproduction of pines. The experimental

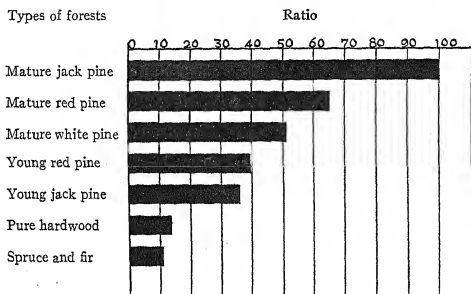


FIG. 17.—Relative light intensity in different types of forest

TABLE V

Forest type	Average difference between thermometer reading	Ratio	Relative intensity in various types
Matured jack pine	1.56	100.0	1.00 (standard)
Matured red pine	1.02	65.3	$2/3+$
Matured white pine	0.80	51.2	$1/2+$
Young red pine	0.62	39.8	$2/5-$
Young jack pine	0.57	36.5	$1/3+$
Pure hardwood	0.215	13.7	$1/7-$
<i>Abies-Picea</i>	0.166	9.6	$1/10+$

records of the Cloquet Forest Experiment Station (25) show that the "no shade" plots of *Pinus Strobus* seedlings developed much better than those of "quarter shade," and the "quarter shade" still better than "half shade." For red pine "one-fourth shade" and "no shade" plots were much superior to those of "one-half shade." General observations on pine reproduction in northern Minnesota

indicate the same situation. In open places where seed trees are available one always finds abundant red pine reproduction (fig. 18), while in a dense stand one can hardly find any. BROWN (6) calls attention to the fact that light is one of the limiting factors for plant succession on the Philippine mountains. In this region, although there are many other factors, the ability to tolerate shade and the light intensity under the forest cover should be counted important factors in determining forest succession.

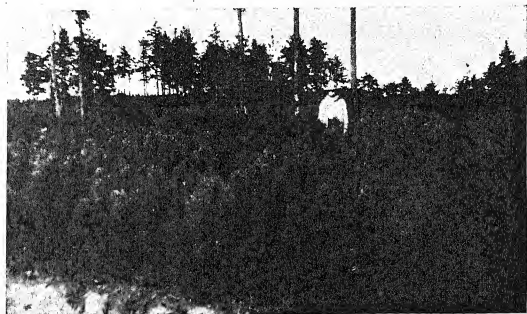


FIG. 18.—Prolific reproduction of *Pinus resinosa* in open near Cloquet, Minnesota

Frequency of seed reproduction.—The frequency of seed reproduction depends upon two main factors, environmental conditions (especially those of soil fertility), and the hereditary character of the different species. Despite the former the difference in the latter may be the limiting factor in determining forest succession. Most of the pines bear seed with definite periodicity. The length of the period is quite irregular, but on the average *Pinus Strobus* has a seed year once in every four, and *Pinus resinosa* once in every seven; while *Pinus Banksiana*, *Abies balsamea*, *Picea canadensis* and a number of hardwoods bear seed almost every year. The infrequency of seed reproduction in *Pinus Strobus* and *P. resinosa* results in a decided advantage to *Acer*, *Abies*, and *Picea canadensis*, and aids them to dominate the succession.

Motility, adaptation, and establishment.—The ability of a species to move out from the parent tree determines its extent of aggregation, and this has a special bearing upon the nature and course of succession. Motility is determined chiefly by the character of the seed and nature of its dissemination; thus seeds of *Populus*, *Acer*, and *Ulmus*, being light and disseminated mostly by the wind, are able to spread much more widely than those of pines. On the other hand, the larger seeds are apt to be carried away by animals to a much greater distance than the lighter seeds are blown by the wind. After the migration of the seeds the ability of different species to adjust themselves to the new habitat determines their establishment. For the adjustment of a plant to new conditions CLEMENTS (8) introduced the term "ecesis," which consists of several processes, germination, growth, competition, reproduction, etc. Here again the three main sets of factors discussed previously have their effect. After all, motility, adaptation, and establishment are important factors to plant succession only during the pioneer stages on areas of denudation, and are of relatively little significance in advanced stages of succession like those existing in this region.

Longevity of trees.—Theoretically speaking, a tree should not die, since a new layer of tissues is always being formed each year. The death of a tree is simply caused by destructive agencies such as fungi, insects, fire, wind, and other unfavorable conditions that reduce the vitality of the tree. The longevity of a tree, therefore, is determined by its ability to resist the destructive agencies and to withstand unfavorable conditions. As a general rule, most of the pioneer species, such as *Populus*, *Betula*, and *Pinus Banksiana*, are very mobile but less resistant to the destructive agencies; consequently they have shorter life cycles than those advanced tree species such as *Pinus resinosa*, *P. Strobus*, and *Acer saccharum*. Those that have the greatest longevity are generally able to dominate the region, but they do not necessarily form the climax of the region, because, as previously stated, the climax of the region is determined not merely by the predominance but by the persistence and continuity of regeneration.

ZOOBIOTIC FACTORS.—These may be divided into two groups, human influences, which consist of cutting, silvicultural treatment,

etc., and the animals and insects. Some of these factors are beneficial and some detrimental to forest succession.

Human influences.—Cutting and silvicultural treatment may bring about progressive succession, or may cause retrogression, depending upon the region and the method of cutting and treatment. In the Adirondack Mountain region, where the virgin forests are mostly mixtures of conifers and hardwoods, the lumberman usually takes out the conifers and leaves the hardwoods. Cutting like this generally advances a stage of succession, because soon after the removal of the conifers the maple reproduction takes place and develops its climax much quicker than would otherwise happen. On the other hand, silvicultural treatment usually takes out the inferior species, such as maple and birch, and makes more room for the pines. Treatment like this generally brings about a mechanical retrogression. Cutting in the region under discussion has been stopped since the creation of the park, and silvicultural treatment has not yet been practiced. Thus for the time being these factors may be disregarded.

Animals and insects.—The effects of animals and insects on forest succession, like those of human influences, may be such as to cause progressive succession or retrogression, depending upon the region and the degree of the influence. In the west overgrazing usually causes retrogression. In the southern pine region grazing generally helps to keep down the undergrowth and encourage the development of the pines, which are generally the climax trees of the region. In the northern woods, during the winter when the ground is covered by snow, deer, rabbits, etc., usually feed on the coniferous seedlings that are covered by the snow. In a region like Itasca, which is under game preservation, these animals are abundant enough to play a part in the limiting factors of forest succession. Insects also may influence forest succession. Among the important insects the spruce budworm may be cited. It has been reported that the last outbreak of this insect was 1910-1919, within which period practically all the spruce and balsam fir within an area of more than 1000 miles from Quebec eastward to the Atlantic were killed back, and birches were left as the dominant tree of the region. A destruction like this would modify forest succession.

In this region, however, neither grazing nor such insect outbreaks seem to have been sufficient to affect succession.

Conclusion

The distribution of the larger plant communities is controlled by climatic factors. The demarkation between the climatic types is by no means sharp, transitional belts of varying widths usually being present. The forest successions within this transitional belt are not controlled by a single factor or by any one of the three sets of factors, but by a complex of factors. In this factor complex the edaphic element, especially the soil composition and soil moisture content, combining with the differences of the evaporating power of the air in different types of forest (figs. 11-13) are the limiting ones in forest succession. The biotic factors, especially tolerance to shade, relative light intensity within each type of forest, and thicket growth of undershrubs which enter into serious competition with tree seedlings for all the necessities of life (figs. 14-16), also play a most important rôle in forest succession. The climatic factors, with the exception of their indirect action through the evaporating power of the air, have very little bearing on the local successions.

Owing to the presence of diversified soil types, a series of edaphic climax types has been, is being, and will be developed. Of these the *Pinus Banksiana* on the sterile sand soils, the *Pinus resinosa* on the sand loam, and the hardwood-*Pinus Strobus* forest on the calcareous clay are the usual types, ameliorated and advanced simply by soil conditions of the region. As time goes on soil conditions are improving, and this would enable a progressive succession to take place or a transformation from one edaphic climax to another. The failure of *Pinus resinosa* and *Pinus Strobus* to regenerate under their own shade, together with the unfavorable conditions of the presence of the thick undergrowth indicates the impossibility of the pines culminating the forest succession of the region. On the other hand, *Acer saccharum* is able to reproduce itself, but is limited to heavy soils. The slowness in the improvement of the sterile sand soils and the limited area of heavy soils in the region make it difficult for *Acer saccharum* to become a climax over the entire area; however, it may develop an edaphic climax and

last longer. The great shade tolerance of the *Abies-Picea* type, with its prolific reproduction on any kind of soil that has adequate soil moisture, as indicated by quadrant study, make it one of the forests best suited to the region. Also, the good water supply, due to the presence of numerous small lakes, favors it to such an extent that it seems both possible and probable that it may develop into the true climax of the region.

Grateful acknowledgment is due to Professors HENRY C. COWLES and GEO. D. FULLER, under whose suggestion and direction the investigation has been conducted. Thanks are due to Professor GEO. D. FULLER for reading this manuscript, and to Dr. HERMAN KURZ for helping to test some of the soil samples. The writer also wishes to extend his appreciation and thanks to the Forest School of the University of Minnesota for their accommodation, and to Professors E. G. CHEYNEY, J. P. WENTLING, C. O. ROSENDAHL, S. A. GRAHAM, and others of the University of Minnesota for their invaluable assistance during the progress of the investigation.

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MEDULLARY BUNDLE IN RELATION TO PRIMARY
VASCULAR SYSTEM IN CHENOPODIACEAE
AND AMARANTHACEAE

CARL L. WILSON

(WITH NINETEEN FIGURES)

The dicotyledonous families Chenopodiaceae and Amaranthaceae are large families of cosmopolitan distribution and somewhat uncertain systematic position. The Chenopodiaceae contain over 70 genera and 550 species; the Amaranthaceae over 40 genera and 450 species. A large number of species, especially of the Chenopodiaceae, are halophytes. These are frequently prominent and occasionally characteristic components of the vegetation of temperate and tropical seashores, of saline soils, of salt marshes, prairies, steppes, and uplands throughout the world. The majority of the Amaranthaceae are tropical and subtropical, but as a whole the two groups seem to be readily adaptable to a wide range of environment. The forms included in these families are nearly all herbaceous, annuals or perennials, only a few woody and treelike forms falling within the group.

The Chenopodiaceae and Amaranthaceae apparently are closely related, having a number of features in common. SOLEREDER (15), in discussing such structures as the simple perforations of the vessels, the pitting in the prosenchyma, etc., as found in Chenopodiaceae, states that there is no decided anatomical character to differentiate them from the Amaranthaceae. HOULBERT (6) likewise places the two families in close proximity. He holds, however, that the Amaranthaceae are more primitive than the Chenopodiaceae, basing his statement upon the nature of the secondary wood. Relationship, moreover, as based upon flower morphology, must be very close. PAX, writing in ENGLER and PRANTL (4), in his discussion of the affinities of the group Centrospermae, indicates the fundamental resemblance of the floral structure in these two families, their floral formulas being practically identical,

and classes them together as one of the four suborders coming from the Phytolaccaceae. A similar opinion is also expressed by Moss (8). SCHINZ, in ENGLER and PRANTL, in discussing the relationships of the Amaranthaceae, holds that the Chenopodiaceae and Amaranthaceae are so closely related that a differentiation between them is nearly impossible, and that the separation into two families is merely one of convenience.

The anatomy of these two families has long been the subject of investigation by numerous writers. The attention which has been

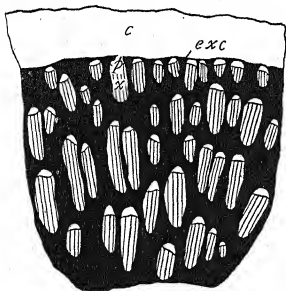


FIG. 1.—Portion of cross-section of stem of *Amaranthus hybridus*: *c*, cortex; *exc*, position of extrafascicular cambium; *p*, phloem; *x*, xylem; black background represents conjunctive tissue.

centered upon the groups in all probability is due to the peculiar character of the secondary growth. The characteristic appearance of a cross-section of a stem of one of these plants possessing some secondary thickening is caused by the manner in which the cambium functions. This commonly results in the appearance of a mass of xylem, in which is imbedded small scattered groups or "islands" of phloem. The tissue, however, in which the phloem is imbedded, although consisting

of cells with thickened and lignified walls, is only in part composed of true xylem. The xylem present is associated with the phloem in such a manner that collateral bundles are formed. The bulk of the lignified tissue surrounding these bundles has been termed conjunctive tissue, which may be either parenchymatous or prosenchymatous in nature. This tissue very commonly becomes lignified shortly after formation by the cambium, thus resembling xylem (fig. 1). This condition is prevalent in all species of these families, with perhaps one or two exceptions, in which there is secondary growth. It is likewise found in the nearly

related families Nyctaginaceae and Phytolaccaceae. Quite universally distributed in the former family, it is found in some few genera of the latter. An homologous condition, according to SOLENEREDER (16), is likewise found in a number of other dicot families and subfamilies, to the number of twenty or more, aside from those mentioned.

In addition to this peculiar type of secondary growth, these families possess another anatomical feature at variance with the usual conditions prevailing in the dicot stem. In several genera the primary bundles of the stem are not arranged in a circle at the periphery of the pith, but are found in the medulla of the stem, separated by several or many cells, of primary origin, from the secondary tissue (fig. 19). In this figure the innermost of the concentric lines represents the position of the extrafascicular cambium, which incloses within it the pith containing the imbedded vascular bundles.

From the time of LINK (7), who was probably the first to publish upon the anomalous secondary growth in these families, research has been directed almost exclusively upon the problems connected with cambial activity and increase in diameter of the stem. These problems deal with such phases of the subject as the relation of the cambium to the primary bundles, and the method of formation and place of origin of the cambium in various species and genera. In general, it may be said that after the formation of the primary bundles, in which (in some forms) a normal cambium may function for some time, a cambium arises in the pericycle. This structure may function in one of two ways: it may continue to produce new tissue during the life of the stem, or it may be replaced successively by the members of a series of concentric cambial rings, each arising, like the first formed cambial layer, in the pericycle. Whether the cambium is permanent or constantly renewed, it

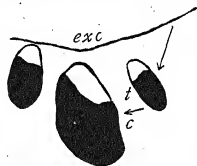


FIG. 2.—*Chenopodium album*: small leaf trace bundle (*l*), after leaving position just under extrafascicular cambium (*exc*), and before fusing with cauline bundle (*c*); both bundles entirely surrounded by primary tissue; direction of movement shown by arrows.

forms, by means of divisions in a centripetal direction, small groups of xylem at intervals, and between these bundlelike groups, interfascicular tissue, the so-called conjunctive tissue. After the formation of a limited amount of xylem, the cambium forms, on its outer face, a small number of phloem cells outside each

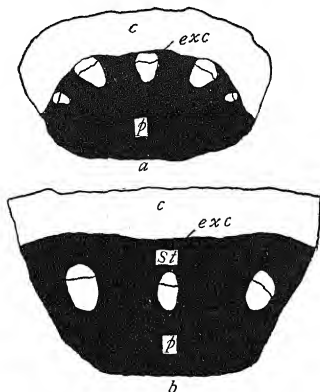


FIG. 3.—Diagram showing apparently medullary bundles: *a*, portion of cross-section of young stem, showing position of extrafascicular cambium (*exc*), in relation to primary bundles, before cambial activity has begun; *c*, cortex; *p*, pith; *b*, position of bundles after some secondary growth; secondary tissue (*st*) cannot be distinguished from cells of pith, and bundles are seemingly entirely imbedded in primary tissue.

group. In the meantime the production of interfascicular tissue on each side continues without interruption. According to ARTSCHWAGER (1) and other workers in this field, the cambium cells within the bundle then cease to function, and a new cambial arc arises in the parenchyma outside the bundle, which becomes connected laterally to the interfascicular cambium. It has also been held that the cambium may be unipolar, that is, producing alternately xylem and phloem in restricted areas, the divisions being entirely in a centripetal direction.

Little work, then, has been done upon the primary growth, with the purpose of acquiring a clear idea of the primary vascular system. The only paper of importance dealing with this phase of the subject is that by FRON (5), who presented diagrams illustrating the primary vascular system in a number of species representing various genera of the Chenopodiaceae. In order that the relationship between the primary vascular tissues and the medul-

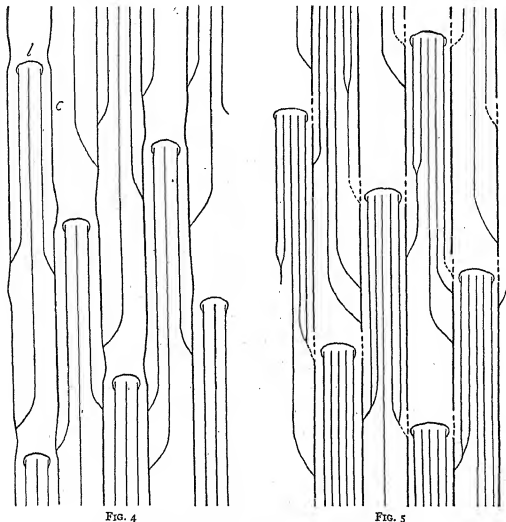
lary bundles in these families may be appreciated and understood thoroughly, as well as that a general idea of the primary vascular tissues be gained, the following series of diagrams is presented.

For the purposes set forth in the foregoing, diagrams representing the longitudinal course of bundles in the stem are certainly necessary; transverse sections in such cases are insufficient for a proper understanding of the course of the primary bundles. Figs. 2-11 were prepared from serial sections of the stems in question, and represent the cylinder, made up of a system of bundles, as split down one side and spread out in one plane. In following the course of the bundles, examination should also be made of the transverse sections shown in figs. 12-19. In all the forms both cauline^{*} and common bundles are shown.

The longitudinal course of the bundles in *Chenopodium glaucum* is shown in fig. 4. The cauline bundles (*c*) are represented by heavy lines; the common bundles by light lines. The point of entrance into the central cylinder of the three bundles making up the leaf trace is indicated by a curved line (*l*). The lateral bundles of the trace pass down the stem for at least two internodes, and fuse with a cauline bundle, one on either side, at some point in the third internode below. The central bundle of the trace traverses four or five internodes before joining a cauline bundle. The leaves are alternate, with a phyllotaxy of two-fifths, which is quite a common arrangement in these families. The cross-section of the stem taken at a node (fig. 12) represents a stage in the growth of the stem at which the extrafascicular cambium has not yet begun to function. The region where this will later develop is represented by the line around the circle of the primary bundles, and in close contact with them. The cauline bundles appearing in this and in succeeding diagrams of transverse sections are indicated at *c*. Two traces having a position at the upper side and lower right

^{*} In the opinion of DE BARY (3), who bases his definitions upon the work of other writers as well as his own, cauline bundles may be considered as bundles found only in the stem. The bundles of the leaf trace may have no direct connection with the cauline bundle, unless, as is frequently the case, they are attached to them laterally. On the other hand, common bundles are structures common to both leaf and stem, forming the bundles of the leaf trace and passing down the stem to fuse with cauline bundles at greater or less distances.

are seen entering the stem in this section, each made up of a large central bundle and two smaller lateral ones. These traces are flanked on either side by cauline bundles, which they join farther down the stem. The other smaller bundles are leaf trace bundles



FIGS. 4, 5.—Longitudinal course of bundles in *Chenopodium glaucum* (fig. 4), and *C. album* (fig. 5).

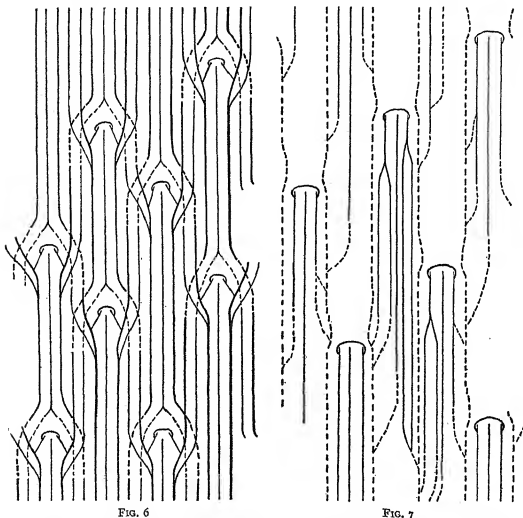
which have not yet fused with a cauline bundle in their downward passage in the stem.

Chenopodium album (fig. 5) resembles the first type very closely. The leaf trace is here formed of five bundles, instead of three, which pass down the stem for a distance of from two to four internodes, occasionally anastomosing, before joining the cauline bundles. In this species, as in most of the forms described here, there is more

or less irregularity in the behavior of the leaf trace bundles in their course down the stem, and some attempt has been made to indicate the nature of this irregularity. While the resemblance in the arrangement of the primary bundles to those of *C. glaucum* is close, a rather remarkable departure from the condition in that species exists. The cauline bundle at the node on either side of the entering leaf trace bends inward into the pith for a short distance, and is therefore separated from the first formed cells of secondary tissue by several (generally four or five) rows of primary cells. This condition is represented in fig. 5 by dotted lines, and in fig. 13 by the position of the large cauline bundles (*c*) at the region of the entering leaf trace. In view of the fact that the cauline bundles traverse the pith, for a short distance at least, they may be said to be medullary bundles for a part of their course. The lateral bundles of the leaf trace on one side, sometimes on both sides, descending from above, and joining the cauline bundle at the nodal region, must also become medullary. This follows from the fact that the cauline bundle is medullary in nature at the point where the trace bundle joins it in its downward course, and the bundle of the trace is therefore obliged to leave the peripheral circle of bundles and pass out into the pith for a short distance, in order to reach the cauline bundle and fuse with it (fig. 2).

The two conditions just described apparently are typical of the divisions made by FRON (5) in his discussion of the primary system of the Chenopodiaceae. The first group includes those forms in which the bundles are always an equal distance from the center of the central cylinder; this type FRON terms rectilinear. The second group includes species in which a certain number of the bundles approach nearer the center than other bundles; this arrangement is called undulate. Forms falling within the first group, according to FRON, are not encountered so frequently as those of the second group. In the first class he placed *Salicornia herbacea*, *Haloxylon articulatum*, *Camphorosma monspeliacum*, *Kochia hirsuta*, *Corispermum canescens*, *Suaeda fruticosa*, *Salsola soda*, *Obione portulacoides*, and several species of *Rhagodia*. In the second group, in which the course of the bundles is undulate, he placed *Atriplex*, *Chenopodium*, *Beta*, *Spinacia*, *Blitum*, and *Roubieva*.

The agreement of this classification of "undulate" (the condition just described in *C. album*), a type where the bundle may be medullary for a part of its course, has been confirmed by the writer for a number of species of *Chenopodium* and *Atriplex*. The condition may not be constant throughout a genus, however. For example,



FIGS. 6, 7.—Longitudinal course of bundles in *Beta cycla* (fig. 6), and *Chenopodium album* var. *anthelminticum* (fig. 7).

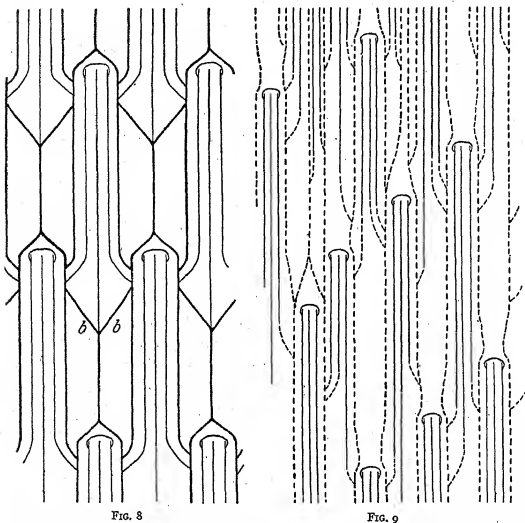
in *C. Botrys* and in *C. rubrum* the cauline bundles pursue a normal course at the node, and this is also true for a nearly related form, *Blitum capitatum*. Moreover, *Spinacia oleracea* examined by the writer has only bundles normal in course.

A drawing copied from FRON, showing the system of bundles in the axis of inflorescence of *Beta cycla*, is shown in fig. 6. The

bundles which are medullary for a part of their course in this form are both cauline and common in nature, as shown by the dotted lines. Again, the bundles of the trace are three in number. The central one splits at a distance of three internodes down the stem from the point of entrance, and the two resulting bundles pass into the pith for a short distance in their downward course (fig. 14). This is a section through a nodal region, and two pairs of bundles having a position in the pith are shown on the right. The outer bundle of each pair is cauline, as indicated. The innermost bundles represent the bundles which have resulted from the splitting of the central bundle of the leaf trace. Each of these leaf trace bundles fuses with the cauline bundle accompanying it two and three internodes farther down the stem. The lateral bundles of the trace to each leaf descend and fuse with a cauline bundle just below the node at which they enter the stem. According to FRON, the course of the bundles is analogous to this form in several other species of the genus, as *Beta maritima*, *B. trigyna*, *B. vulgaris*, etc.

In *Chenopodium ambrosioides* var. *anthelminticum* the course of the bundles is very erratic, but the fundamental scheme of arrangement is probably as shown in fig. 7. Of the three bundles to the trace, the lateral ones divide immediately upon leaving the stele, indicating that a possible fusion of bundles may be going on. The central bundle of the trace descends a number of internodes (four or five) before fusion with a cauline bundle, which is entirely medullary in this species. The lateral bundles of the trace likewise descend a number of internodes, as many as three or four, and a considerable amount of fusion and anastomosing takes place before these bundles finally join the cauline bundles on either side. As in *C. album*, the leaf trace bundles likewise become medullary, in this case frequently for some distance, before joining the cauline bundle. In fig. 15, a transverse section of the same stem, the three bundles of one trace are seen at the top of the diagram just after entrance into the stele. Five cauline bundles are shown, all of them separated from the extrafascicular cambium by several rows of cells of primary origin. The remaining bundles consist of bundles of trace which will fuse with cauline bundles farther down.

In *Amaranthaceae* it will be seen that *Iresine paniculata* presents a very regular arrangement of primary bundles (fig. 8). Fig. 16 represents a cross-section of the stem, taken somewhat below the middle of an internode. The three bundles making up the leaf trace descend in the stem side by side, and fuse with stronger



FIGS. 8, 9.—Longitudinal course of bundles in *Iresine paniculata* (fig. 8), and *Celosia cristata* (fig. 9).

cauline bundles at a point about two internodes from the node at which they enter. In this species large cauline bundles, represented in fig. 16 by the two large opposite cauline bundles, split immediately above the entrance of a leaf trace into the stem, and the two branches descend on either side, thus closing the leaf gap. Descending to the next node, these bundles fuse with the cauline

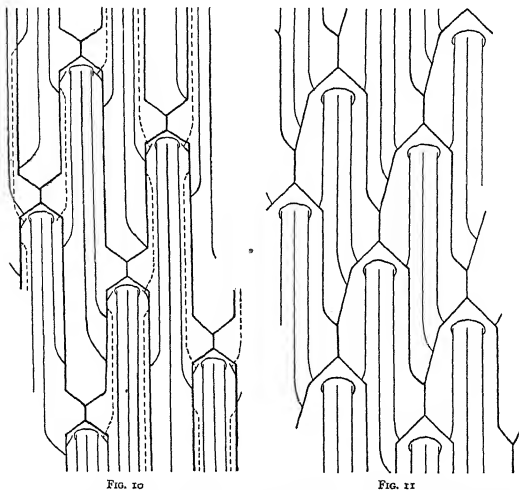
bundles subtending the leaf trace. The four smaller cauline bundles seen in fig. 16 are the branches resulting from the splitting, above the leaf gap, of the large cauline bundles at the next node above. Branches (*b*) from the bundles subtending the trace form the large cauline bundles, and at the point where these fuse they are joined by the central bundle of the trace descending from above.

The condition in *Celosia cristata*, as shown in figs. 9 and 17, is very similar to that in *Chenopodium ambrosioides*. There are many more primary bundles in the stem, and their course is even more irregular than in this latter species. A cross-section through the nodal region is shown in fig. 17, with a leaf trace made up of bundles irregular in size and shape, shown entering the stele at the top of the diagram. In a section which shows the beginning of secondary growth, the cauline bundles throughout their course are again seen to be separated from the secondary tissue by a few rows of cells of primary origin. The fundamental arrangement of the bundles in this species agrees so closely with that of *C. ambrosioides* that a detailed description is not presented.

In connection with this species, attention should be called to a possible misinterpretation of structure in the stem. The cells of the first formed secondary tissues do not become lignified, either in this case or in a large number of other species in these families. At least the change, if it does occur, does not come about until comparatively late in the life of the stem. These cells become so disposed as to resemble primary tissue: They remain thin-walled, and no distinct radial arrangement is to be observed, the arrangement of the cells being like those of the pith which they adjoin. Later formed tissue becomes lignified and thick-walled, and apparently represents the cells first formed by the cambium. The primary bundles thus appear to be entirely medullary, when in reality they are bordered on the outer side by secondary tissue. In forms like these, therefore, in which the cambium is commonly extrafascicular, the primary bundles appear to be, as DE BARY applies the term, "apparently" medullary (fig. 3). Sections of very young stems prevent any misunderstanding in this respect. This condition, while prevalent in many forms of this group, is

particularly striking in *Celosia*, and attention is therefore called to it at this point.

Several points of difference from preceding forms are shown in *Amaranthus graecisans*, in figs. 10 and 18. In the species last described, it was shown that only certain of the bundles had a position in the pith either entirely or throughout a part of their

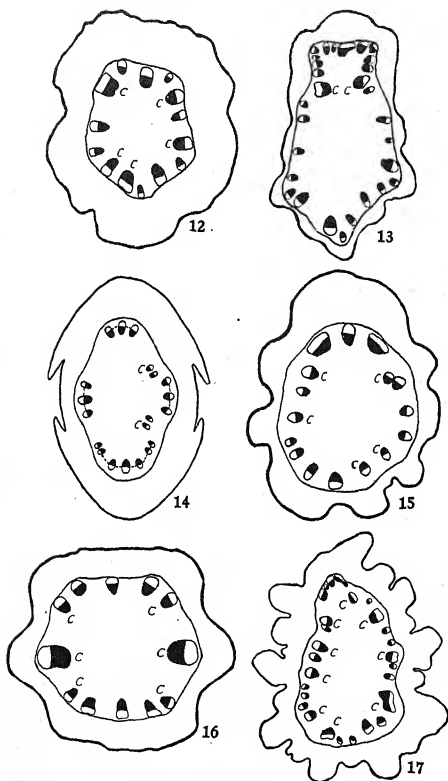


FIGS. 10, 11.—Fig. 10, longitudinal course of bundles in *Amaranthus graecisans*; fig. 11, *A. hybridus*, showing course of inner circle of bundles represented in fig. 19.

course in the stem. In the form now under discussion, it can be seen (fig. 18) that all of the primary bundles are separated from the region of secondary growth, and imbedded in primary tissue throughout their course in the stem. Another point of difference lies in the fact that the bundles are arranged roughly in two concentric circles. The innermost of these circles is formed by

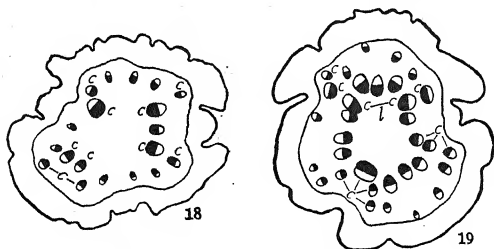
cauline and common bundles, the outer is formed of cauline bundles alone. Fig. 18 represents a cross-section of the stem taken somewhat below the nodal region. At the top three bundles of the trace are seen entering the stem, flanked on either side by cauline bundles. These are properly a part of the inner circle of bundles, but have not yet progressed far enough inward to this position. The cauline bundles of the outer circle are intimately connected with the corresponding bundles of the inner circle. In fig. 10 these cauline bundles of the outer ring are shown by dotted lines. They are seen to take their origin from the large cauline bundles of the inner circle at a region just below the node, pass out of the inner ring and down the stem two or three internodes, finally fusing with a complex of bundles which arises at each node. In most cases this fusion seems to be with a branch trace, just after the trace enters the stem. In effect, the result is a fusion with practically the same elements from which the bundle takes its origin. These outer cauline bundles quite frequently divide once after passing to the outer ring, and on the other hand occasionally fail to appear at all. The small bundles of the inner circle are leaf trace bundles which have not yet fused with the large cauline bundles on either side in their downward course. The leaf gap is closed in the same manner as in *Iresine paniculata*.

In *Amaranthus hybridus* (fig. 19) there are seven, mostly well developed bundles to the leaf trace, as compared with three (occasionally five), as seen in *A. graecizans*. The four outermost of these bundles remain in the outer ring, and together with the outermost cauline bundles make a more definite system of circles. At each node a few bundles leave the peripheral circle of tissue near the entering leaf trace, and pass into the pith, fusing with branches from the large cauline bundles (connected by the line *l* in fig. 19) of the inner ring on either side of the entering trace. The four small outermost bundles of the trace fuse with the group of bundles thus formed, all the bundles ultimately joining the large cauline bundles previously referred to, on each side of the trace. There is thus seen to be a connection between the peripheral circle of bundles and the medullary system of bundles. The course of the bundles is again apparently very irregular, and various conditions



FIGS. 12-17.—Stem cross-sections at nodal region: fig. 12, *Chenopodium glaucum*; fig. 13, *Chenopodium album*; fig. 14, *Beta cyclo*; fig. 15, *Chenopodium ambrosioides* var. *anthelminticum*; fig. 16, *Iresine paniculata*; fig. 17, *Celosia cristata*.

prevail. For instance, one of the outermost of the leaf trace bundles may descend the stem several nodes, and then pass into the peripheral circle of bundles, so that the cambium of the bundle is continuous with the extrafascicular cambium. The bundle would thus again connect the medullary system with the peripheral secondary growth. It is unnecessary to discuss such irregularities at length. The fundamental plan of the primary system in these two species appears to be the same, although that of *A. hybridus* is the more complex. This is due to the fact that there are more well defined bundles to the trace, and also that the cauline bundles



FIGS. 18, 19.—Stem cross-sections at nodal region: fig. 18, *Amaranthus graecizans* fig. 19, *A. hybridus*.

of the outer circle always divide into two and sometimes three bundles before proceeding down the stem. The complexity of the stem is such that a diagram showing the course of all the bundles at once would be difficult to interpret. For this and for other reasons to be brought out later, a diagram has been prepared (fig. 11) showing the longitudinal course of the bundles of the inner circle only, as seen in a transverse section of the stem. The diagram is thus made up of cauline bundles and the three innermost bundles of the leaf trace. These latter pass down the stem and fuse with the cauline bundles in the usual manner. The leaf gap is closed in the same manner as in *Iresine* and *A. graecizans*. Based upon the material so far examined, such species as *A. blitoides* agree

with the condition in *A. graecizans*. Other species, such as *A. retroflexus*, *A. gangeticus*, *A. salicifolius*, *A. Torreyi*, and *A. caudatus*, accord with the condition in *A. hybridus*.

Discussion

MEDULLARY BUNDLE

Of all the departures from normal structures in the dicot stem, the medullary bundle is one of the most striking. It occurs in a large number of unrelated families, perhaps thirty or more. The exact number of families in which it is found is difficult to determine, because of the confusion of terms applied by various writers. The term "medullary bundle" is quite commonly applied to intraxylary phloem, better known as internal phloem, which is present in a number of dicot families. In such cases, groups of protoxylem occasionally lie closer to the phloem than to the metaxylem, and hence appear to accompany the former rather than the latter. This further adds to the confusion, since there thus appears to be present a ring of medullary bundles. According to our present conception of internal phloem, however, these detached portions are phylogenetically portions of the vascular ring of bundles.

WEISS (18) and COL (2), who appear to be the chief contributors to the subject of true medullary bundles, in studying these structures in various families, found that medullary bundles are generally common bundles, rarely cauline. They do not ordinarily constitute an independent system in the stem, but are in connection with the peripheral circle of bundles at the node, and are most frequently an evident and direct prolongation of the normally situated bundles.

So far as the writer has been able to find, but little attempt has been made to account for this departure from the normal fundamental type of dicotyledonous stem. COL (2), in attempting to account for the condition in various families with which he worked, found a crowding at the node due to the large number of bundles coming in from the leaf and floral regions. He therefore claimed that since no space was available in the periphery, the bundles were crowded into the pith and cortex. It would seem pertinent to quote SINNOTT (14) in this connection:

Against the argument that medullary bundles in ferns are caused by the influence on the stele of the complication of the petiolar bundle, it may be pointed out that in the Marattiaceae the intricate vascular systems of stem and leaf communicate with one another by only a double trace, and that in young plants of *Pteris* a medullary bundle appears in the stele, while the leaf trace is still a very simple strand.

WESTERMAIER (19) mentioned several species of *Begonia* in this connection. These species are perennial by tubers or rhizomes. According to this writer the amount of translocation taking place in such a plant, as compared with woody forms, makes necessary the development of other bundles in the pith to supplement those of the normal ring of bundles. PETERSEN (10), in his discussion of internal phloem in *Campanula*, describes transition stages between internal phloem and medullary bundles. Several forms have internal phloem closely associated with the peripheral ring of bundles or in isolated groups. In another case medullary phloem was found with which xylem was associated. A transition is thus built up to other species with well developed medullary bundles. It is probable that PETERSEN had in mind a phylogenetic development of medullary bundles in this genus, although this is not certain from the context of his paper.

In whatever manner such conditions arose, it is difficult to conceive a situation in which they arose suddenly to the form in which we now observe them. A series of steps, we must believe, has intervened between some generalized form and the present apparently specialized condition. Further, our present conception of the origin of many plant structures leads to the belief that such conditions must have had a long evolutionary history, analogous to the history of other plant structures which have been studied by various workers in recent years. Evidence of a geological nature in support of this may be somewhat lacking, but this does not necessarily invalidate the conception. It has become almost axiomatic that the origin of a species or group is not necessarily indicated by the earliest records of their discovery. ZEILLER (20) states that the earliest fossil remains of herbaceous species of any of these families are fruits of a species of *Salsola* in the Tertiary of Europe. Leaf impressions of various species of *Pisonia*, a genus

of the nearly related family Nyctaginaceae, have been found in various formations of the upper Cretaceous in this country and Europe. *Pisonia*, however, is a woody genus. It would seem, therefore, that so far as geological evidence goes, the Chenopodiaceae and Amaranthaceae may be of somewhat recent origin. As previously stated, however, this evidence should have little weight, and the complexity of the groups as seen in the medullary bundle and type of secondary thickening would suggest much specialization and greater age.

In spite of structural similarities, it is highly probable that the medullary bundle has arisen independently in the various groups in which it is found. This is made all the more probable when one considers the totally unrelated families in which the structure is prominent, as the Piperaceae and the Campanulaceae. This conception is supported by SCOTT and BREBNER (13) in their explanation of the origin of anomalous secondary growth in the Iridaceae. This growth bears a remarkable resemblance to the same type of growth as found in Liliaceae. They say "it is very probable that the first origin of secondary growth may be taking place today, just as we find medullary bundles appearing in certain dicots as an individual peculiarity."

The medullary bundle is not a structure of wide distribution in the two families treated here. The type of bundle arrangement as seen in *Amaranthus* was first noted by LINK (7), and apparently is widely distributed, if not universal, in the genus. Medullary bundles were reported by REGNAULT (12) as occurring in *Chenopodium ambrosioides*. VOLKENS, in ENGLER and PRANTL (4), recorded their presence in *Acroglochin persicarioides*. This last writer states that there are eight cauline bundles in the stem of this plant.

Assuming that such an arrangement of bundles as seen in *Amaranthus* is the highest type in these families, the species of the two families so far studied have been arranged in a manner which seems to be in accord with a possible method of origin of the medullary bundle in these, or in other groups. The view is here advanced that the medullary bundle has arisen by the gradual advance of bundles from the peripheral circle of bundles into the pith. This

change has most probably originated at the node, and gradually extended up and down the stem. The influence of any two successive nodes gradually makes itself felt throughout the internode. It is highly probable, according to the evidence presented here, that the cauline bundles were foremost in this inward progression. This statement is based upon the species just described, such as *Chenopodium album*, *C. ambrosioides*, and *Celosia cristata*, in which the cauline bundles have advanced into the pith, either at the node only, or for the entire length of the bundle. The common bundles remain at the margin of the pith, in connection with the extrafascicular cambium. That this is probably not the usual order of evolution is shown by the fact, already stated, that medullary bundles as a whole are said to be leaf traces.

Chenopodium glaucum and *Iresine paniculata* may be taken as representing a more generalized type of dicot stem. The first departure in the direction of a specialized condition may be found in such a form as *Chenopodium album* (figs 5, 13), in which, at each nodal region, the cauline bundles pass into the pith for a short distance, quickly returning to their peripheral position. The influence of the node, if such it may be termed, becomes more powerful in such a species, as shown in figs. 6 and 14, copied from FRON. Here this influence operates to such an extent that the divided leaf trace bundle on either side of the node is affected equally with the cauline bundle on each side, and both bundles pass into the pith. This is indicated by the dotted lines in fig. 6, and by the two medullary bundles on each side of the entering leaf trace at the right in fig. 14. As in the preceding form, this medullary course of the bundles is of but short duration, and the two bundles in their downward course quickly return to their former position. *Chenopodium ambrosioides* (figs. 7, 15) exhibits the next step in this possible progression, in that the cauline bundles are wholly medullary, although as yet very close to the extrafascicular cambium. The common bundles, however, remain in connection with the extrafascicular cambium for a considerable distance before moving into the pith to join the cauline bundles. This form, however, may be taken to represent a stage near the culmination of this evolved condition, at least in this family.

Turning now for evidence to the Amaranthaceae, *Celosia cristata* is seen to be quite similar as regards the course of the bundles to the form just described. This species, therefore, may be taken to represent a stage at the same level of development in this family which *Chenopodium ambrosioides* represents in the Chenopodiaceae. This suggests that the medullary position of the bundles may have evolved independently in the two families, since homologous stages in development may be found in each family.

Amaranthus is used in this paper to represent the highest point of development of the medullary condition, at least in these two families. This is supported by the fact that all primary vascular bundles are entirely medullary, and the only connection with the tissue at the periphery of the pith takes place at the nodal region. Here again it would seem that the cauline type of bundle has probably been foremost in this inward movement of bundles, since these are found lying deepest in the pith. It is true that branches of these bundles pass into the outer ring of bundles, but this might be held to be due to the influence of the nodal region, where these changes take place.

Some significance may be attached to the diagram shown in fig. 11. Here, as previously described, only the inner circle of bundles seen in fig. 19 is incorporated, no connections to the outer ring of bundles being shown. A fundamental resemblance to the other species can be seen in the manner in which the leaf gap is closed, in the course of the trace bundles, and in the manner in which they join the cauline bundles. In view of these facts, such a species is held to be a derived form, so far as primary anatomy goes.

It is not intended that the idea developed in the foregoing be a conclusive statement as to the method by which the medullary bundle has evolved in all the dicot families possessing that structure. It is a possible method by which this type of structure may have originated and developed in these groups in particular, and possibly in other families. The medullary bundle, then, is a structure occurring in a number of dicotyledonous families, the significance and function of which is unknown. The origin and history of development of such a disposition of bundles are debat-

able, however, and considerable speculation concerning it would be permissible, if for no other reason than that of stimulating further work of a comparative nature in this direction.

PRIMARY VASCULAR SYSTEM

Some attention should first be given to the taxonomic value of such structures. In order that use may be made of plant structures in deciding affinities, it is evident that there must be available a large body of knowledge in the field. In using such characters as the nature of the primary vascular system to this end, the worker is handicapped by the lack of information available on the subject. Of the small amount of research which has been carried on in this direction, nearly all has been done by the earlier students of plant anatomy. In this connection should be mentioned the pioneer work of NÄGELI (9), who figured a number of diagrams of primary vascular systems, which have been extensively copied by textbook writers since his day. DE BARY (3) reviewed the facts as known to him, and since that time but few papers have appeared containing material of this type. Since little is known about the primary anatomy in general of our great families and alliances, it is difficult to evaluate and make use of such evidence from the standpoint of determining relationships.

In the families concerned here, it appears, at least from the comparatively few genera about which information of this kind is available, that the primary anatomy might possibly be significant in this direction. The great majority of investigated species have a bundle system made up of both cauline and common bundles. They thus depart from what has been called the dicotyledonous type, in which all the bundles of the stem are leaf trace bundles. The presence of both types of bundles is not universal, however. The writer has found that in the cases of *Gomphrena globosa* and *Spinacia oleracea* all the bundles of the stem are common bundles. It has already been stated that FRON presented diagrams which illustrated the primary vascular system in a number of species of Chenopodiaceae. Several of these, which represent various genera, possess a leaf trace of but one bundle. This condition is probably due to reduction, as the species in which it occurs are

halophytes. A comparison can be drawn, however, between the species FRON figures, in which no indication of reduction occurs, and the diagrams presented here. The general impression is reached that the arrangement of the primary bundles offers significant evidence as to the close relationship of these families. This conclusion is reached chiefly from the fundamental arrangement of the bundles shown. The chief indications rest upon the relation of the cauline and common bundles. The species figured by FRON which show indications of reduction may be included in this comparison. Fundamentally their arrangement is the same as the other species considered. It is doubtful what emphasis should be placed upon the number of bundles of the trace, as these vary. The number of cauline bundles, too, appears to be variable. It might seem desirable to proceed farther and in greater detail in this comparison, but lack of a wider knowledge of primary systems in these and other families makes the matter difficult. Little or no information is available which would show that a certain type of primary structure is constant throughout a family. From the material presented here, it would seem that a definite arrangement of primary bundles is characteristic of these two families.

The family Dioscoreaceae possesses a type of primary vascular system which merits our attention at this point. First, however, a word should be said with reference to an anomalous type of secondary thickening found not only in this family, but also in a number of genera belonging to several other families of the Liliales. The fact has already been mentioned that this condition is almost exactly paralleled by the peculiar type of cambial activity found in the families treated in this paper. The general appearance of the tissues, and the manner in which they are produced, is fundamentally the same in both groups. Several early writers, including SANTO (17), have called attention to this, although so far as the writer is aware there have been no important discussions calling attention to a possible phylogenetic interpretation of this condition. A superficial observer, however, taking these facts, together with the monocot stemlike arrangement of the primary bundles in *Amaranthus*, might speculate upon the relationships of the Amaranthaceae and the Liliales. For this reason it has seemed best to

discuss briefly some of the factors having to do with relationships in these groups.

According to DE BARY, the dicotyledonous type of primary system is characterized chiefly by the fact that the vascular system of the stem is made up only of bundles of the leaf trace. This he says characterizes not only the majority of the dicots, the Coniferae, Gnetaceae, Osmundaceae, and *Equisetum*, but likewise Dioscoreaceae. With the exception of this family, the primary anatomy of the members of the Liliales which possess anomalous secondary growth is of the monocotyledonous type. Later investigations, however, have shown that the Dioscoreaceae do not belong in the class in which they were placed by DE BARY. The researches of QUÉVA (11) have shown that the primary anatomy of the family as a whole is characterized by being made up of both cauline and common bundles. He figures diagrams of the course of the bundles in longitudinal view which, although simpler, closely resemble the diagrams shown in this paper. The node is trilacunar, however, that is, each of the three bundles of the trace causes a gap of its own as it leaves the stele. The Chenopodiaceae and Amaranthaceae are mostly unilacunar, that is, but one gap is left in the stele as the leaf trace leaves the stem, no matter how many bundles make up the trace. The writer has found, as a single exception to this, that in *Chenopodium Bonus-Henricus*, which possesses five bundles to the leaf trace, there are three gaps left by the departing vascular supply to the leaf. The significance of this is doubtful, and at any rate cannot be evaluated until more is known of primary systems in general. For the present, the appearance of the characters under discussion, although present in both monocots and dicots, had better be regarded as a question of parallel and independent evolution.

Summary

1. An anatomical study has been made of the primary vascular system of a number of species of Chenopodiaceae and Amaranthaceae.
2. A series of diagrams of the longitudinal course of the primary bundles in these species is presented. They set forth in an ascending series a probable method of evolution of the medullary

arrangement of bundles as found in *Amaranthus*, and possibly as found in other dicotyledonous families possessing medullary bundles.

3. It is concluded that the steps in this progression are possibly as follows: (a) the cauline bundles on either side of an entering leaf trace pass into the pith a short distance (probably due to the influence of the node), and return quickly to their normal position just under the extrafascicular cambium; (b) this condition becomes exaggerated: the cauline bundles abandon their temporary course in the pith, and become medullary throughout their course in the stem; (c) the bundles of the trace behave in a similar manner. In the earlier stages of such a development, they are also members of the peripheral ring of bundles; later their course in the stem becomes more and more extended, until they are finally completely medullary.

4. Attention is called to the belief, based chiefly upon the wide distribution of medullary bundles among various dicotyledonous families, that this bundle arrangement has probably had an independent evolution in the various groups in which it is found.

5. Based upon the nature of the primary vascular system, the two families dealt with here are considered to be closely related. This conclusion, based chiefly upon flower morphology, has already been reached by other workers.

The writer wishes to express his gratitude to Professor ARTHUR J. EAMES of Cornell University for his suggestion of this problem, and for his helpful advice and kindly criticisms throughout the progress of the investigation.

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GROWTH OF CORN AND SUNFLOWERS IN RELATION TO CLIMATIC CONDITIONS*

W. F. HANNA

(WITH FOUR FIGURES)

Introduction

Within recent years an extensive literature has developed relating to the nature of the growth rate in plants. Various hypotheses have been advanced, but scarcely any of them offer a full explanation of the phenomena of growth; indeed, in many cases the conclusions reached are quite contradictory.

BLACKMAN (1, 2) and REED and HOLLAND (13) have laid emphasis upon the "internal" factors which govern plant growth. They have evolved growth formulas, which, in the cases investigated, seem to explain fully the course of the plant's development, independently of any fluctuations in climatic conditions. BRIGGS, KIDD, and WEST (3, 4, 5, 7, 8, 14) have undertaken an extensive study of the whole subject of growth rate, dealing especially with the plant processes which in any way might be responsible for the particular form of the growth rate curve.

Numerous investigations have also been carried out with a view of bringing out the relationship between growth rate and such "external" conditions as temperature. Probably the best work on temperature alone has been done by LEHENBAUER (9), working with maize seedlings. In a recent monograph LIVINGSTON and SHREVE (10) have given an extensive review of the subject of environmental conditions and plant growth, and also have suggested and employed various indices which promise to be of value in the study of climate and its relation to plant growth.

In introducing new agricultural plants to any particular district, the questions of growth rate and the effect of different climatic factors in accelerating or retarding growth become of particular

* Report of an investigation carried out in the Department of Field Husbandry, University of Alberta.

interest. The sunflower, for example, has been recently introduced into northern districts as a forage crop, and in some respects is considered to be superior to corn. There is, however, a lack of exact knowledge as to the relative climatic adaptation of these two crops. In order to obtain such information, the following study was undertaken. Records of growth measurements, yields, and climatic conditions were kept. By a careful analysis of the data thus obtained it was hoped to derive fundamental information regarding the growth adaptations of these crops which might throw light upon their suitability to various parts of the country.

Experimentation

This work was carried out during 1920 and 1921. The general method employed was that of seeding standard varieties of *Helianthus annuus* and *Zea Mays* at a number of dates, beginning quite

TABLE I
PLAN OF SEEDING, OBSERVATIONS, AND YIELDS FOR 1920

Date of seeding	Plot	Crop	Date of appearance above ground	Days from seeding to appearance	Date harvested	Actual yield per plot (lb. green weight)	Percentage dry matter	Corrected yield per acre (tons dry matter)
May 14....	1	Corn	June 1	18	September 20	134.6	15.8	1.95
May 14....	2	Sunflower	May 25	11	September 20	350.7	14.2	4.33
May 21....	3	Corn	June 7	17	September 20	139.7	14.9	1.69
May 21....	4	Sunflower	June 4	14	September 20	368.2	14.8	4.33
May 31....	5	Corn	June 14	14	September 20	154.6	18.4	2.08
May 31....	6	Sunflower	June 10	10	September 20	405.4	14.1	4.19
June 10....	7	Corn	June 18	8	September 20	144.8	15.8	1.53
June 10....	8	Sunflower	June 17	7	September 20	387.5	14.3	3.77
May 14....	9	Corn	June 1	18	September 20	177.8	17.9	1.95
May 14....	10	Sunflower	May 25	11	September 20	451.5	15.1	4.33

early in the spring, and continuing until about the first week in June. The varieties used were Mammoth Russian sunflower, and Northwestern dent corn. The plans of seeding, observations, and yields are presented in tables I and II.

In order to measure the growth in height, ten plants were selected in each plot, and a small wooden stake was driven into the soil beside each plant, the top of the stake being level with the

surface of the ground. The sunflowers were measured from the top of the stake to the apex of the stem. In 1920 the corn was measured from the top of the stake to the highest point of the plant. With this method, however, the height was found to fluctuate somewhat with the turgidity of the leaves. This difficulty was overcome in 1921 by stretching the leaves of the corn plants upward to their full extent, and measuring from the top of the stake to the tip of the longest leaf. In 1920 measurements were taken at three-day intervals; in 1921 at two-day intervals. Figs. 1 and 2 show the mean height of the ten plants in each plot at different dates.

TABLE II
PLAN OF SEEDING, OBSERVATIONS, AND YIELDS FOR 1921

Date of seeding	Plot	Crop	Date of appearance above ground	Days from seeding to appearance	Date harvested	Actual yield per plot (lb. green weight)	Percent age dry matter	Corrected yield per acre (tons dry matter)
May 10*....	1	Corn	May 25	15	September 3	707.5	15.3	2.95
May 20.....	2		June 4	15	September 3	599.5	15.8	3.02
May 30.....	3		June 8	9	September 3	726.0	16.4	3.27
June 9.....	4		June 17	8	September 3	693.5	13.9	2.67
May 16*....	5		May 25	15	September 3	654.0	16.2	2.95
May 10.....	6	Sunflower	May 23	13	September 15	549.0	20.3	2.94
May 20.....	7		June 1	12	September 15	399.0	22.9	2.46
May 30.....	8		June 3	9	September 15	502.0	21.6	2.98
June 9.....	9		June 17	8	September 15	424.0	20.4	2.43
May 10.....	10		May 23	13	September 15	484.0	21.1	2.94
May 3.....	11		May 18	15	September 15	588.0	21.7	3.75

* Corn of plots 1 and 5 killed by frost on morning of May 28, and seeded again May 30; plants of second seeding appeared above ground June 8.

For the purpose of calculating the actual growth rates presented in fig. 3 for 1920, the height of the plants in plots 1, 3, and 9 of corn and plots 2, 4, and 10 of sunflowers have been averaged for each date of measurement. In 1921 the actual growth rates indicated in fig. 4 have been obtained from similar averages of plots 1, 2, and 3 of corn, and plots 6, 7, and 10 of sunflowers. The relative growth rates shown in figs. 3 and 4 have been calculated from these averages by employing BLACKMAN'S (1) compound interest formula: $\frac{R}{100} = \frac{\log_{10} H_2 - \log_{10} H_1}{t \times 0.4343}$, where H_1 is the height

of the plant at the beginning of the period, H_2 the height at the end of the period, t the length of the growing period (in 1920

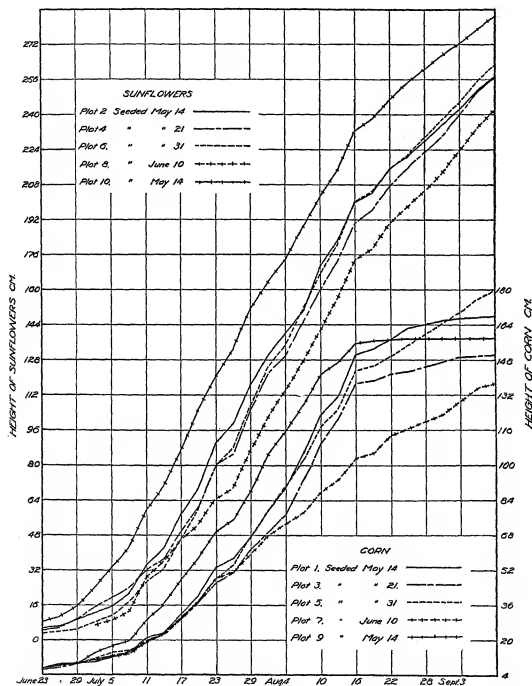


FIG. 1.—Mean height (cm.) of corn and sunflower plants at different dates in 1920

3 days, and 1921 2 days), and R the percentage of increase in height per day, per unit of height, for the whole period.

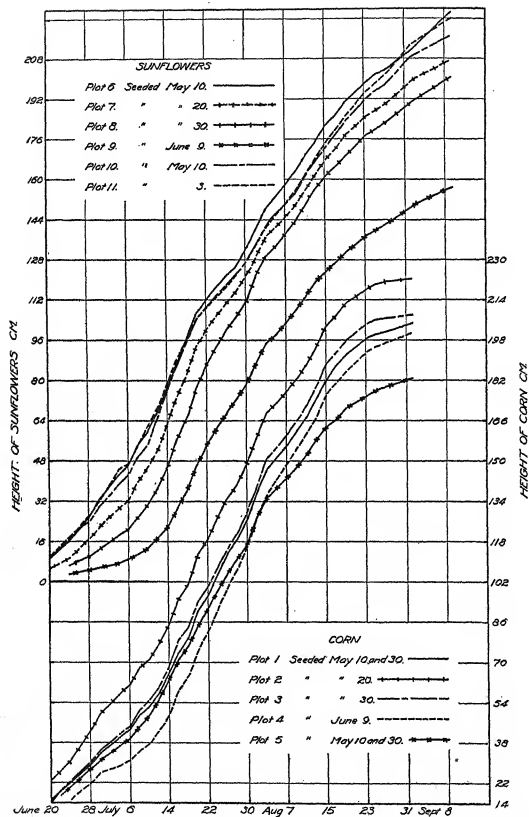


FIG. 2.—Mean height (cm.) of corn and sunflower plants at different dates in 1921

When the plots were harvested the green weight of fodder was obtained. At the same time a representative sample was taken from each plot and the percentage of dry matter determined. The corrected yields per acre, given in tables I and II, were calculated by the method outlined by NEWTON (12).

Temperature data for the two years were obtained from a self-recording atmospheric thermograph situated close to the plots. From these charts the mean bi-hourly temperatures have been obtained. The mean temperature for each day of the growing season was then calculated from the bi-hourly readings. In this way it has been possible to arrive at a series of values which are based not only upon the magnitude of the temperature, but also upon its duration.

Reference was made to the work of LIVINGSTON and SHREVE (10) in order to express temperatures in the form of the following efficiency indices for plant growth.

(1) Direct temperature efficiency indices.—This method assumes that above 0°C . the rate of plant growth is directly proportional to the temperature.

(2) Remainder temperature efficiency indices (growth at 4.5°C . taken as unity).

(3) Remainder temperature efficiency indices (growth at 10°C . taken as unity). The remainder indices are similar to the direct indices except that in each case a higher temperature has been assumed as the zero point of growth.

(4) Exponential temperature efficiency indices.—The calculation of these indices is based on the assumption that at 40°F . plant growth takes place at unit rate, and that for each increase of 18°F . (or 10°C .) above this point the rate of growth is doubled, according to the Van't Hoff-Arrhenius principle.

(5) Physiological temperature efficiency indices.—LEHEN-BAUER'S (9) data on the growth of maize seedlings at various temperatures when exposed for twelve-hour periods have been used by LIVINGSTON in preparing these indices. Plotting growth rates as ordinates, and temperatures as abscissae, a curve was obtained giving indices ranging from zero at 2°C ., through a maximum of 122.3 at 32°C ., to zero again at 48°C .

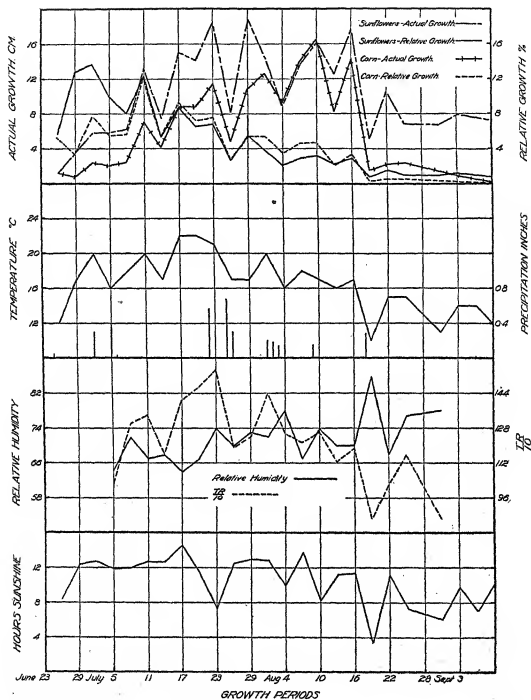


FIG. 3.—Actual and relative growth of corn and sunflower plants for three-day periods in 1920, together with mean temperature (C.), precipitation (inches), mean relative humidity, the product $\frac{TR}{10}$, and mean daily hours of sunshine for corresponding periods.

(6) Degree hours.—This method of expressing temperature indices has been used by MOSIER (11) in his study of the climate of Illinois. Considering the zero point of growth as 40° F., degree hours have been calculated for the different periods by multiplying the number of hours during which the temperature is above 40° F. by the number of degrees the average temperature is above that point.

Records were also kept of relative humidity, precipitation, and total hours of sunshine. In 1921 the maximum solar radiation was obtained from a black bulb thermometer *in vacuo*, for each day from July 4 to September 8. As high temperatures combined with high relative humidity should give conditions most favorable for rapid growth, the value $\frac{TR}{10}$ has been employed in this study as an attempt to express this condition. Here T is the mean temperature (C.) for the period of growth, and R the mean relative humidity for the same period. The large products resulting have been divided by 10 in each case. The voluminous tables of data recorded or calculated for the foregoing measurements and indices are not included in the present brief summary of the investigation. These, however, have been utilized in deriving various coefficients of correlation, some of which are reported later.

Discussion

From an examination of tables I and II, it is evident that the interval of time from seeding to the appearance of the plants above ground decreases with each date of seeding. While in the early May seedings, sunflowers have germinated much more rapidly than corn, the last seedings show little difference in this respect. No soil thermograph records were kept during 1920, but the readings for 1921 show that the soil temperature increased from 53° F. on May 14 to 65° F. on June 9. Sunflower seeds, therefore, seem to germinate with little difficulty at low temperatures, while corn germinates much more rapidly as the temperatures increase. This fact has an important bearing upon the date of seeding corn in northern districts. In 1921, by delaying the seeding from May 10 to May 30, the time required for germination was shortened

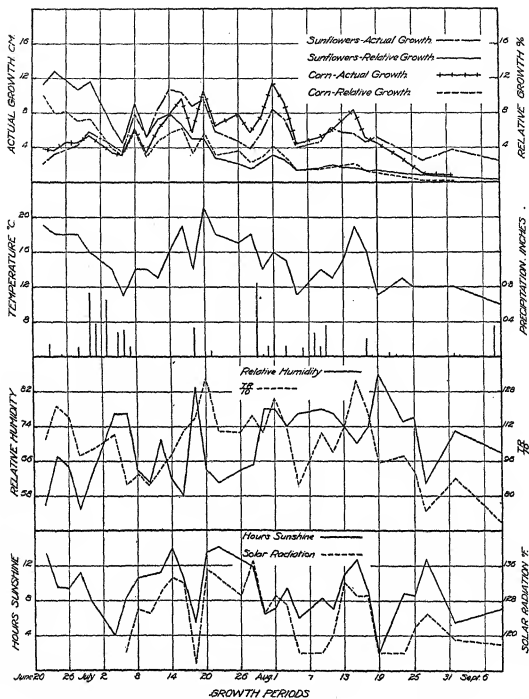


FIG. 4.—Actual and relative growth of corn and sunflower plants for two-day periods in 1921, together with mean temperature (C.), precipitation (inches), mean relative humidity, the product $\frac{TK}{10}$, mean daily hours of sunshine, and mean maximum solar radiation (F.).

by six days. In 1920 the temperature dropped to 29° F. on the morning of June 13, doing considerable injury to the first and second seedings of corn. These plants recovered, however, and a second seeding was not necessary. The sunflowers were not noticeably injured by this frost. Corn seeded on May 31 escaped this frost, as it did not appear above the ground until June 14.

In 1921 the first seeding of corn was killed by frost on May 28, when the thermometer registered 24° F. It was necessary to reseed plots 1 and 5. In this year, therefore, the earliest seeding of corn to escape frost injury was that of May 20. The sunflowers, while not killed by the frost, seemed to have suffered injury. Many plants assumed a branching form, while several of the leaves were later seen to curl slightly.

The yields of sunflowers in 1920, given in table I as tons of dry matter per acre, are about double those for corn during the same year, notwithstanding the higher percentage of dry matter in the corn. In 1921 the sunflower yields were greatly reduced, while the yields of corn increased. During the two years considerable variation in precipitation was recorded. The precipitation from September 1, 1919, to August 31, 1920, totaled 21.08 inches, with 9.15 inches falling during May, June, and July. In 1921 records for corresponding periods show a precipitation of 15.65 inches and 7.48 inches respectively. It may be concluded, therefore, that sunflowers have a relatively high water requirement. This is in harmony with results obtained by WOLLNY. In his work, reported by BRIGGS and SHANTZ (6), the water requirements of corn and sunflowers are given as 233 and 490 respectively.

From the preceding it is evident that sunflowers germinate more rapidly in the early spring than corn, and survive temperatures as low as 24° F. without serious injury. Their growth, however, is quickly retarded in dry soils.

In figs. 3 and 4 the actual and relative growth rates have been plotted, together with the climatic data for the same periods. During both years the relative growth rate is quite high at the beginning of the season, but gradually falls, and finally assumes an almost straight line, parallel to the time axis. Both actual and relative growth rates show a closer relationship with temperature

than with any of the other climatic factors. The corn seems to have made especially rapid growth at high temperatures.

Since the relation between temperature and growth seems to be so intimate, it becomes of interest to consider the coefficients of correlation for a few of the plots. The coefficients of correlation with the direct temperature efficiency indices for each date of seeding of corn and sunflowers are given in table III.

TABLE III
COEFFICIENTS OF CORRELATION (r) FOR DIRECT INDICES AND GROWTH

Date seeded	Plot	Corn	Plot	Sunflowers
1920				
May 14.....	1	0.3613±0.1173	2	0.5184±0.0987
May 21.....	3	0.3389±0.1104	4	0.4184±0.1113
May 31.....	5	0.3479±0.1186	6	0.4729±0.1047
June 10.....	7	0.4793±0.1108	8	0.2864±0.1320
1921				
May 10.....			6	0.6496±0.0689
May 20.....	2	0.4564±0.0959	7	0.4854±0.0911
May 30.....	3	0.4000±0.1017	8	0.5427±0.0869
June 9.....	4	0.4814±0.0963	9	0.4778±0.0951
May 3.....			11	0.6655±0.0664

In both 1920 and 1921 the correlations between the rate of growth of corn and changes in temperature as measured by direct indices are very slight, especially when considered in relation to the probable error. There seems to be, however, a slight increase in the correlation value as the date of seeding becomes later, the last seedings giving the highest correlations. On the other hand, with sunflowers the early seedings have given the best correlations, while the values for the later dates fall off decidedly.

In order to measure, if possible, the accuracy with which various other indices express the relationship between climatic conditions and growth, correlation coefficients have been calculated for plot 2 (1921) of corn, and plot 11 (1921) of sunflowers. These plots were chosen because they represent the earliest successful dates of seeding for the year. In addition, they gave evidence of corre-

lation with the direct indices, a form of temperature measurement commonly employed in such studies. The results are given in table IV.

The best correlation between the growth of sunflowers and temperature is obtained when direct temperature indices are employed. With the remainder indices above 10°C. , and also with the physiological indices, there is no correlation whatever. The relation with hours of sunshine and the product $\frac{TR}{10}$ is very slight, while the value for relative humidity is a minus quantity.

TABLE IV
COEFFICIENTS OF CORRELATION (R) FOR PLOTS 2 AND 11 (1921)

Efficiency indices	Plot 2 Corn	Plot 11 Sunflowers
Direct indices.....	0.4564 \pm 0.0959	0.6655 \pm 0.0664
Remainder indices (4.5°C.)..	0.5292 \pm 0.0872	0.6469 \pm 0.0693
Remainder indices (10°C.)...	0.6407 \pm 0.0714	0.2571 \pm 0.1113
Exponential indices.....	0.4582 \pm 0.0957	0.6387 \pm 0.0706
Physiological indices.....	0.5798 \pm 0.0804	0.0355 \pm 0.1190
Degree hours.....	0.5380 \pm 0.0861	0.6207 \pm 0.0733
Hours sunshine.....	0.4322 \pm 0.0985	0.4100 \pm 0.0992
$\frac{TR}{10}$	0.6606 \pm 0.0683	0.3991 \pm 0.1002
Relative humidity.....	-0.0910 \pm 0.1201	-0.0600 \pm 0.1188

The correlation between the growth of corn and temperature is considerably better with the remainder indices of 4.5°C. than with direct indices. It is still higher with remainder indices of 10°C. , while the best correlation with the growth of corn has been obtained with the product $\frac{TR}{10}$. The correlation between the growth of corn and physiological indices is comparatively good when compared with the corresponding value for sunflowers. Since the physiological indices were actually derived from LEHENBAUER'S (9) data on maize seedlings, it is not surprising to find that the corn plants in their growth show a much closer relationship with these indices than do sunflowers. It appears, therefore, that physiological temperature efficiency indices for expressing plant growth can be of little value unless derived from observations on the particular kind of plants under consideration.

Although too much reliance cannot be placed upon the correlations in table IV, there seems to be considerable evidence to show that while sunflowers make good growth at medium or quite low temperatures, corn responds most closely to high temperatures, and is particularly influenced by favorable conditions of temperature and relative humidity.

From a study of the climatic data, growth measurements, and yields for the two years, certain conclusions may be drawn regarding the climatic adaptations of these two crops. The outstanding difference in the climate for the two seasons was the low precipitation for 1921, which was accompanied by a light yield of sunflowers. Corn on the other hand showed no such reduction in yield. Sunflowers have germinated and started growth more rapidly than corn when seeded at the early dates; they have not been injured seriously by late spring and early fall frosts, and for that reason have a considerably longer growing season. Corn has been very susceptible to frost injury, and gives evidence of rapid growth at high temperatures.

Sunflowers, therefore, should be better suited than corn to those parts of the country where precipitation is ample, but where there is a short growing season with low temperatures in spring and fall, and danger of frost injury. Corn should give good results where the season is longer, temperatures higher, and where soil moisture is not sufficient for the successful culture of sunflowers.

Summary

1. During 1920 and 1921, a study has been made of the growth of *Zea Mays* and *Helianthus annuus* in relation to their climatic environment. The varieties used were Northwestern dent corn and Mammoth Russian sunflowers.

2. Early seedings of sunflowers were found to germinate and appear above ground sooner than the early seedings of corn, while the late seedings of both plants required about the same time for this purpose. Both were retarded by the low soil temperatures obtaining early in the season, but the corn to a greater degree than the sunflowers.

3. A temperature of 29° F. in the spring of 1920 injured the corn but did not affect the sunflowers. In 1921 a temperature of 24° F. killed the corn and slightly injured the sunflowers.

4. Because of resistance to late spring and early fall frosts, sunflowers have a longer growing season than corn.

5. An effort has been made to correlate the growth of corn and sunflowers with five temperature efficiency indices used by LIVINGSTON and SHREVE, as well as with degree hours, total hours of sunshine, the product $\frac{TR}{10}$, and mean relative humidity.

6. The growth of both corn and sunflowers has shown closer correlation with temperature than with any other single climatic factor. Corn gave the best correlation with temperature when remainder indices derived from temperatures above 10° C. were employed. Sunflowers gave the best correlation with temperatures above 0° C. This indicates a greater capacity in sunflowers than corn for growth at low temperatures.

7. Physiological temperature efficiency indices derived from LEHENBAUER'S observations on the growth of maize seedlings have shown no relation to the growth of sunflowers, but have given a distinct correlation with the growth of corn.

8. Lack of sufficient soil moisture in 1921 was undoubtedly responsible for the low yields of sunflowers in that year. Corn showed no reduction in yield due to this cause.

9. Corn promises to give better yields of fodder than sunflowers in areas where the growing season is comparatively long, temperatures are high, and soil moisture is limited. Sunflowers should be well suited to sections where moisture is more abundant, temperatures at the beginning and end of the growing season low, and where there is danger from spring and fall frosts.

The writer wishes to acknowledge his indebtedness to Dr. ROBERT NEWTON, of the University of Alberta, under whose direction the work was undertaken and carried out.

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ORIGIN AND DEVELOPMENT OF VASCULAR SYSTEM OF LYCOPODIUM LUCIDULUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 323

JAMES JESSE TURNER

(WITH SEVEN FIGURES)

Comparatively few original papers have been published which deal with the anatomy of the sporophyte of *Lycopodium*. Of these, the later ones deal mainly with the structures of the fully developed stele, and only the earlier ones have discussed the origin and development of the tissues. Occasional brief references have been found, and these will be considered in their proper connection in the body of the paper.

In view of these facts, it has been the object of this investigation, in so far as was possible, to bring our knowledge of the origin and development of the vascular tissues, of at least one species, up to date. *Lycopodium lucidulum* Michx. was chosen as representative of the more primitive group of the genus. This species is too familiar to need any description, except to say that the alternating groups of sporophylls and vegetative leaves enable one to make a fairly accurate estimate of the age of any particular portion of the stem. The material for the investigation was supplied from northeastern Ohio by Professor JESSIE M. JEROME of Hiram College.

Stele

As early as 1846, NÄGELI (7), in his researches on the growing apices of plants, examined the apex of *L. clavatum*. He figures a very definite apical cell. He noted the simultaneous differentiation of the woody strands and the leaf traces, and concluded, not without considering other possibilities, that each strand passes off into a leaf trace. He refuted the impression, then current and still accepted in some places, that the vascular cylinder consists of a single bundle, and pointed out that it was made up of as many bundles as there are centers of lignification. In 1855 CRAMER

(2) studied the apex of *L. Selago*, and pointed out that there is no relation between the number of leaves in a single turn of the spiral and the number of strands making up the stele. In 1872 HAGELMAIER (3) published a series of articles in which he discussed the anatomy of the stem and the development of the apical region. His conclusion was that there is an apical group of from two to four cells that give rise to tissues of the stem. That same year STRASBURGER (8) gave an account of the apical development of *L. Selago*. His conclusion was that a single apical cell gives rise to the dermatogen and the perilem, and that one or two deeper cells give rise to the plerome. Later (9) he states that all the tissues of the stem arise from a single apical group. He figures three initials in transverse section and two in longitudinal section. Since that time, four papers have appeared on the anatomy of different species of *Lycopodium*, but they offer no considerable contribution to our knowledge of the development of the tissues (4, 5, 6, 10).

In the present investigation of *L. lucidulum*, the writer has never been able to trace the origin of the stem tissues to a single apical group, much less to a single apical cell. At the center of the apex there is a surface group of cells, numbering from three to five or possibly more, that by their activity give rise to the dermatogen and the perilem. Immediately below these is another group of about the same number which gives rise to the plerome. It is with this last group that we are concerned in the study of the origin of the stele. They divide both by anticlinal and by periclinal walls, but the anticlinal divisions are much more numerous at first. As a result, a lengthwise section of the cylinder shows a very blunt apex. The differentiation of the tissue systems is very rapid; 0.5 mm. below the extreme apex of the stem, it is possible to distinguish the groups which give rise to xylem from those which give rise to phloem. It seemed best to give names to these groups, and I have called the former prexylem and the latter prephloem. At this same level, differentiation is evident in the individual prexylem groups. The cells from which the protoxylem differentiates are much smaller and stain more deeply than those from which the metaxylem differentiates. On the other hand, those cells which, from their position, must be prephloem cannot be distinguished from the pericycle.

Throughout the entire period of development, the differentiation of the phloem lags behind that of the xylem. In material which was fixed in November, the older portion of the last summer's growth showed the first protoxylem well lignified, while the pre-phloem groups can be distinguished from the pericycle only by a slight difference in the intensity of the stain (fig. 1).

The usual number of xylem groups is six, although there are numerous variations, ranging from five to eight, both in the same

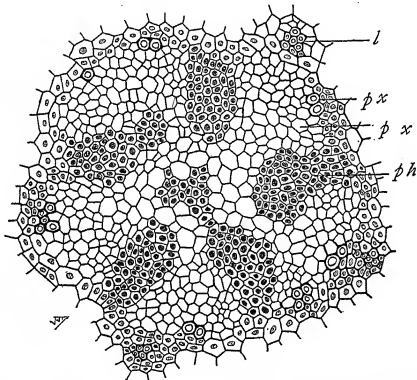


FIG. 1.—Cross-section through last summer's growth: *p-x*, prexylem; *ph*, phloem; *px*, protoxylem; *l*, leaf trace.

stem at different levels and in different stems. In some slides of sporelings, kindly loaned me by Mr. E. A. SPESARD, the xylem groups range from two to four.

The arrangement is typically radial, the xylem groups alternating with phloem groups, and meeting more or less completely in the center. Sometimes a mass of phloem occupies the central portion. This, however, always connects with one of the peripheral bands, although in particular sections it may show as an isolated group. The differentiation of both xylem and phloem is centripetal. In my opinion the stele should be considered as a truly radial stele and not as a gamostele, either phylogenetically or ontogenetically.

In the early part of the second year, the remaining protoxylem lignifies and elongation practically ceases. The actual number of protoxylem elements is very small, if by protoxylem is meant elements with spiral or annular thickenings. From a study of developing tissues, it would seem that there is no fixed line between protoxylem and metaxylem, but that the particular type of thickening is merely a function of elongation. CHAMBERLAIN (1) calls

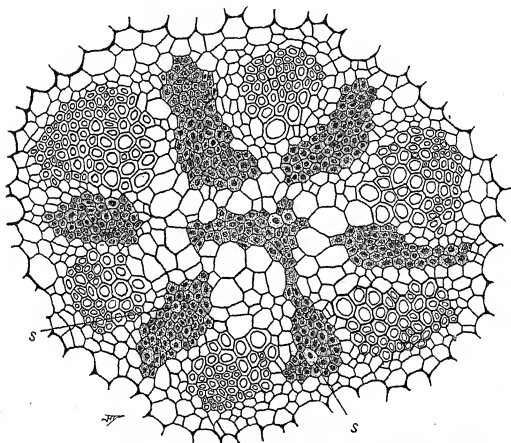


FIG. 2.—Cross-section through portion of stem two years old: lignification of xylem well along; differentiation of phloem beginning; *s*, differentiating sieve tube.

attention to pitted tracheids in what may fairly be called the protoxylem of the sporelings of *L. scariosum*. By the end of the second year, from two-thirds to three-fourths of the metaxylem is lignified. At this time occasional cells in the phloem groups show, by the lighter staining of the cytoplasm and nuclei, that they are differentiating into sieve tubes. I was not able to demonstrate the presence of any fully developed sieve tubes with sieve plates higher in the stem than the three-year old portion (fig. 2).

In the third year the lignification of the xylem is completed, and fully developed sieve tubes appear. In this particular species of *Lycopodium*, by far the larger part of the metaxylem consists of pitted tracheids; the smaller ones with uniseriate circular pits, the larger ones with oval multiseriate pits. Scalariform tracheids occur, but they are few in number. It is probable that the scalariform tracheids are a modified form of the pitted tracheids, in which

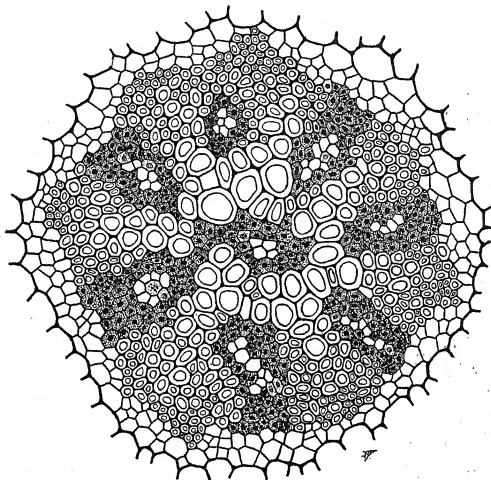


FIG. 3.—Section through three-year old portion of stem, showing completed stele

the pits are extremely elongated. Tracheids were seen which were pitted at one end and scalariform at the other, with a transition between the two forms of thickening.

The sieve tubes occupy a narrow strip in the central portion of the phloem. I could not determine that there was any definite order in their differentiation. Sometimes the first tube appeared close to the outer edge of the phloem, and sometimes close to the center of the mass. The sieve plates are scattered over the walls, and vary greatly in size (fig. 3).

Leaf strand

The leaf primordium starts as a single cell. This cuts off lateral cells and then a basal cell, but the resulting leaf is not wholly a product of these cells. The initiation of growth in the primordium seems to act as a stimulus to the surrounding tissues. Cells are activated on all sides, extending as far on the outside as the base of the preceding leaf. As a result, at the apex of the stem the leaves succeed one another without any visible length of stem intervening. It is only by the later elongation of the stem that the leaves are separated. This accounts for the decurrent leaf bases of the mature leaves (fig. 4).

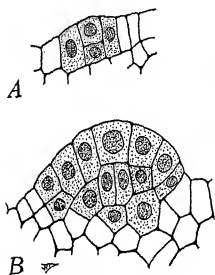


FIG. 4.—Leaf primordium: *A*, first divisions; *B*, involving of deeper layers in development of leaf.

The activation proceeds downward from the base of the developing leaf, involving deeper and deeper layers of cells. The progression in this direction is much slower, and does not reach the stele until some time after the desmogen strand is well differentiated in the leaf base (fig. 5).

The differentiation of the desmogen strand, by repeated longitudinal divisions without the transverse divisions which occur in the adjacent cells, is first evident in the base of the leaf primordium. The progression is, from this time on, both acropetal and basipetal. Acropetally, it just about keeps pace with the growth of the leaf; it never quite reaches the apex. The tip of the mature leaf is without vascular elements. Basipetally, the differentiation of the desmogen strand progresses toward the desmogen strand of the stele, meeting it some distance down the side, at an acute angle. The primordium arises without any reference to the location of the protoxylem groups. In the downward differentiation, if the desmogen strand happens to be opposite a phloem group, it swings to one side and joins with the adjacent protoxylem group. The lignification of the foliar strand proceeds in the same way.

The first thickening appears in the cells of the leaf base. By the time the first vessel has joined with the stele, lignification has begun

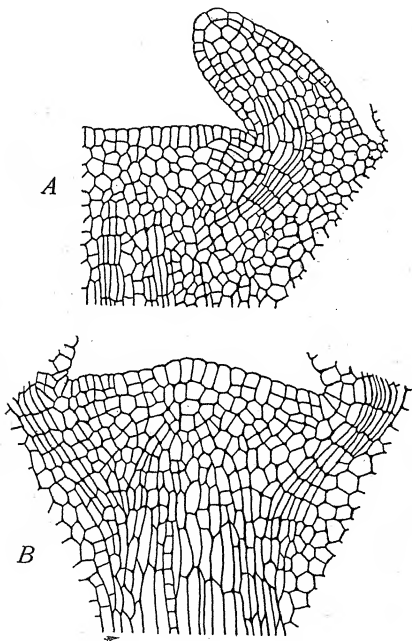
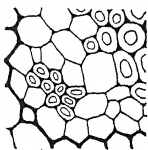


FIG. 5.—Developing strand of leaf: A, strand in leaf base only; B, connecting of strand with stele.

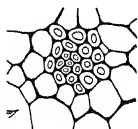
in other elements in the leaf base. Considerably more lignification takes place in the leaf and upper portion of the leaf trace than in that part of the trace which makes connection with the stele. A

cross-section of the leaf trace well out in the cortex shows about twice as many lignified cells as the same trace after it has passed through the endodermis of the stele (fig. 6).

The differentiation of the bundle itself begins in the center. The first lignified cell does not always occupy the exact center of the bundle, but always, so far as could be observed, within one or two cells of it. The lignification proceeds outward about equally in all directions, and is complete before the end of the first year.



A



B

FIG. 6.—Two sections of same leaf trace: A, just inside endodermis of stele; B, 0.5 mm. outside endodermis and 1.5 mm. higher up in stem.

All of the lignified elements have spiral thickenings. In macerated preparations some of these were found to be true vessels and others tracheids. Some of these tracheids were very short, being about ten times as long as wide. No cells could be found in the leaf bundle proper or in the leaf trace that could properly be called phloem. In leaves less than one year old there was no well defined endodermis, but there was a sharp line of demarcation between the tracheids and the abutting cells. In the two-year old leaves the endodermis was well defined. In leaves older than this the thickening of the adjacent cells was extended from one to two cells farther out. The endodermis is continued in the leaf trace,

but disappears as the trace passes through the endodermis of the stele (fig. 7).

Discussion

At the present time there are two views regarding the number of categories which should be made of the parts of the plant body. The older view, and the one most generally held, is that the plant body should be divided into roots, stem, and leaves. The more recent view is that the plant consists of roots and leaves. This latter ground is taken on the interpretation of the stele as being composed of leaf traces. In other words, the stem is made up of aggregated leaf bases. Whether this interpretation is justifiable

in phyllosiphonic plants, I cannot say. The scope of this investigation does not permit of an answer, but probably those who take this view disregard the telescoping of the stem which is so common in the higher plants. From this study of *Lycopodium* as a representative of cladosiphonic plants, however, I am convinced that this interpretation for this group is not supported by the facts. In *Lycopodium* the very primitive radial stele is mapped out in

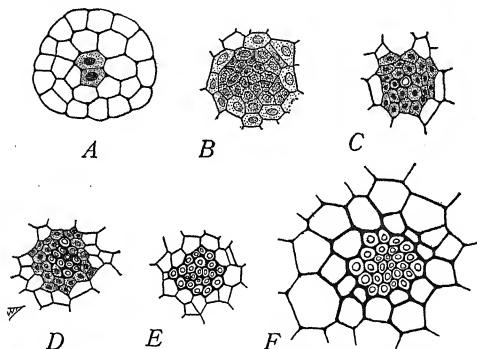


FIG. 7.—Cross-sections of leaf bundle in different stages of development: *A*, beginning of desmogen strand in primordium; *B*, desmogen strand practically completed; *C*, beginning of lignification, showing mesarch character of bundle; *D*, bundle partially lignified, showing outward progression of process; *E*, bundle completely lignified but with no endodermis; *F*, bundle from two-year old leaf with well defined endodermis.

the sporeling from the beginning, and continues to develop from an independent group of meristomatic cells so long as the plant continues to grow. This continuous stele is interrupted only by branches, and these originate by a division of the independent apical group. The vascular system of the leaves originates independently of the stele, and connects with it only by a differentiation of cortical elements. *Lycopodium*, at least, has a stem.

On the other hand, it seems that too much stress has been laid on the necessity of exactly delimiting the categories. They should

be considered as composite parts of a unified whole, rather than as separate parts making up a whole. To illustrate, the major part of the leaf trace, in its origin, belongs to the stem; but through differentiation it becomes, to all intents and purposes, an integral part of the foliar system. It may be considered as either stem or leaf, or as both stem and leaf. The same may be said for the outer layer of the cortex. By origin, it is stem; by activation, due to the formation of leaf primordia, it becomes leaf; by subsequent elongation, it again becomes a part of the stem. Where does it belong?

The direction of the differentiation in the leaf trace is a subject which deserves more attention than has been given to it. No positive statement has been found in the literature that the differentiation proceeds in either direction. Various writers speak in a vague way of the leaf trace entering the stele or passing out into the leaf; apparently the use of the terms depended upon the subject under discussion. When speaking of the stele, the leaf trace passes out; when speaking of the leaf, the trace passes into the stele. The fact is the trace passes neither in nor out. The cells composing it are laid down in place as a result of divisions in meristematic cells. The progress of the differentiation of these cells may be either in or out. In *Lycopodium* the differentiation begins in the leaf base and passes in toward the stele. What it may do in the other groups of plants is not clear from any evidence discovered in the literature on the subject.

Summary

1. The central cylinder originates independently of the leaf traces from a group of meristematic cells situated just below the apex.
2. The central stele is an exarch radial protostele consisting of alternating plates of phloem and xylem.
3. There is no sharp differentiation between the protoxylem and metaxylem, the later-forming protoxylem having the structural characters of metaxylem.
4. The differentiation of the phloem proceeds much more slowly than that of the xylem.

5. The desmogen strand of the leaf originates independently, from a group of cells in the base of the leaf primordium. Only at a later period does it join the stele by a differentiation of cortical elements.

6. The foliar bundle is mesarch, and is made up exclusively of spiral elements.

The writer wishes to express his appreciation and gratitude to Dr. W. J. G. LAND for advice and criticism, and particularly for his kindly encouragement during the progress of the investigation.

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CLOGGING OF STOMATA OF CONIFERS IN RELATION TO SMOKE INJURY AND DISTRIBUTION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 324

J. B. RHINE

Introduction

When an area of vegetation is invaded by coal smoke, the first of the higher plants to succumb to its destructive effects are the conifers. This has been attributed to the fact that the conifers are exposed all the year, and that the smoke is usually worst during the winter. In view of the fact, however, that conifers are more susceptible than other evergreens, and the deciduous European larch is more sensitive than deciduous foliage trees, mere length of exposures will not explain the situation. The explanation which has received the most support is that this higher susceptibility of the conifers to smoke injury is due to the deeply sunken stomatal pits and their tendency to catch soot and tars, thus becoming clogged. This claim was made by BAKKE (1), who attempted to estimate quantitatively the foreign material present by washing the needles with ether. He states as follows:

On account of their deeply sunken stomata the conifers are more susceptible to smoke injury. . . . In cross-sections of conifer needles from a smoke region the stomata were found to be at least partially filled with a tarry compound.

The most extensive work supporting this theory is that of the Leeds group, investigating smoke injury in the neighborhood of that city. RUSTON and CROWTHER (10) reported that conifers were especially sensitive to smoke. They stated that some leaves had as high as 80 per cent of the stomata blocked by soot and tar. COHEN and RUSTON (3) and CROWTHER and STEUART (5) referred to the clogging effect of soot on evergreen vegetation. RUSTON (9) stated that "80 per cent of the stomata of firs grown in the city of Leeds were found clogged by tar." COHEN and RUSTON (4) showed drawings of the stomatal pits of fir and juniper needles

with heavy black deposits overlying the guard cells. SMALLWOOD (12) found over 85 per cent of the stomata of pines growing in Washington Park, Chicago, were clogged by soot by the first of August.

In view of these reports, the claim of CLEVINGER (2) that only a small percentage of the stomata are clogged in smoke injury cannot be considered correct. He found, however, as did STOCKHARDT (13), that applications of soot alone to young firs did not produce any apparent injury to them, although slight injury was caused to the older needles of young Jack pines.

Investigation

With this work in mind, Dr. WILLIAM CROCKER, Director of Boyce Thompson Institute for Plant Research, suggested an investigation of the internal physiological condition resulting from this clogging of the stomata by soot. The work was undertaken in the fall of 1922. Smoke injured needles were taken from Austrian pines growing in Washington Park, Chicago, cross-sections made of them, and these, mounted in water, were examined microscopically.

It was found at once that every stoma was clogged, that all were overlain by a black, tarry-looking mass which certainly looked like foreign material collected in the deeply sunken stomatal pits. Even when the chief part of the deposit had been carried away by the sectioning blade, examination showed particles still adhering to the walls of the pit, indicating that the black deposit had once been there. The clogging substance filled at least half of the stomatal pit in the case of the Austrian pine, although if the section were thick it appeared to fill the whole pit. Curiously enough, however, the black color disappeared when alcohol was added to the slide, and small bubbles passed off at the same time, leaving the deposit a very light brown. This was found to be soluble in ether and xylol. Other material was then examined which had not been exposed to smoke, such as greenhouse grown conifers, nursery stock, needles collected in the mountains, etc., and the black deposit was found to be present in all, regardless of habitat. It had to be concluded, therefore, that the substance under investigation was not soot, tar, or any other foreign material, but instead was a natural product of the conifer itself.

Further examination of the literature with the new situation in mind revealed the fact that the presence of this natural deposit in conifer stomata had been noted as early as 1827 by LINK (6), had been reported by SCHLEIDEN (11), had been investigated to a considerable extent by ZUCCARINI (18), THOMAS (14), WILHELM (16), MAHLERT (7), and WULFF (17), and was mentioned in such texts as PFEFFER's *Pflanzenphysiologie* and HABERLANDT's *Physiologische Pflanzenanatomie*.

LINK, SCHLEIDEN, and THOMAS called the deposit a resin, while ZUCCARINI, WILHELM, MAHLERT, and WULFF claimed it was a wax. Alcohol was the reagent upon which these conclusions were based, in the main. It is not surprising, therefore, that there should be some disagreement, since it is difficult by this means alone to distinguish between wax and resin microchemically. There are more modern tests, however, that readily distinguish between waxes and resins. Chloral hydrate will dissolve resins but will not dissolve waxes. Concentrated sulphuric acid will dissolve resins, producing a brown color, but will not dissolve waxes (15). These tests were made on the stomatal deposits of Austrian pine, two species of juniper, and three of fir. In all cases examined the deposit was found to be a wax and not a resin. It appeared to be the same substance that occurs on the under side of the needles of many conifers; and in the needles which had both the wax deposit in the stomata and the coating on the surface of the leaf, the two seemed continuous. This was first pointed out by WILHELM.

The wax appears granular under high magnification, and is evidently porous, since air bubbles are seen to escape from the deposit when alcohol is added to a cross-section previously mounted in water. This air is not driven out by cold water, because of its lower penetrative power, and the air having a lower refractive index than the water appears black, and gives the wax granules the appearance of soot. Furthermore, the need for this route as a channel for gas exchange in the conifer needle does not allow any other conclusion than that the wax is penetrated by gases.

While gas exchange is possible through this deposit of wax, undoubtedly it is very much retarded. The wax must form a "damper" on the processes of respiration, transpiration, and

photosynthesis, protecting the tree against excessive water loss and reducing the rate of metabolism. If this be true we should expect that trees having this protecting wax would be able to live in more xerophytic conditions, while those lacking it, other things being equal, would be confined to the more moist regions. It is true that we cannot determine to what extent "other things are equal," and therefore cannot ascertain the exact value of this stomatal deposit as a factor in conifer distribution, but there can be no doubt that it is a factor of considerable importance.

In support of this view, the following interesting parallel between xerophytism and the presence of stomatal wax in the conifers is pointed out. The conifers reported to have wax in their stomatal pits are *Abies* spp., *Picea*, *Juniperus*, *Cedrus*, *Thuja*, *Pinus*, *Cupressus*, *Chamaecyparis*, *Larix*, and others of the Abietineae and Cupressineae. These wax-containing conifers for the most part are xerophytic, and either inhabit dry regions or the "physiologically dry" conditions of northern latitudes, where the frozen soil of winter affords very little moisture. *Abies* and *Picea*, which advance farthest into this latter condition, have very heavy deposits of wax. The piñon pine of the arid southwest likewise has an extremely large amount of stomatal wax. On the other hand, the following conifers, which are well known as moist habitat trees, and which are confined to moist temperate or tropical rain-forest conditions, do not have the deposit of wax in their stomatal pits: *Araucaria*, *Dammara*, *Podocarpus*, *Torreya*, *Taxodium*, and *Taxus*. *Ginkgo*, as a close relative, might be mentioned in this group as not having the wax. *Pinus cubensis* was found to be without the stomatal deposit. We can hardly avoid the conclusion, therefore, that this wax deposit affects the moisture requirement, and consequently the distribution of the conifers that have it.

Since the stomatal deposit is not soot, the relation between smoke injury and the conifer stomata is not so obvious. In fact, there is much question whether there exists any such relation at all. PFEFFER (8) thought the "resin" (he referred to the work of THOMAS) might catch soot and dust, and hold enough foreign material on its surface to close its pores. It is true that the wax deposit in the stomata of needles exposed to dust and smoke does

have a thin film of foreign substance over its outer surface, and this film does not disappear in alcohol. In fact, the whole surface of the needle becomes coated with soot during the winter in smoky regions. The addition, however, of more particles to the granular wax deposit could only make the retarding layer a trifle thicker, at worst. If the air pollution includes tarry and oily substances, it might well be that in time these would close the pores of the sievelike layer of wax, but the adhesive particles would certainly clog the tiny stomatal slits themselves, where there is no wax overlying, more readily than the larger expanse of wax. Moreover, if such clogging were the nature of the injury, STOCKHARDT and CLEVINGER should have obtained injury to fir trees from their heavy applications of soot, especially since fir has much wax.

As a factor affecting the moisture relations of the conifers, however, the stomatal wax may have some bearing on their higher susceptibility to smoke injury. It is well known that the trees most resistant to smoke injury are the willow, cottonwood, elm, elder, alder, sycamore, tulip tree, catalpa, white poplar, aspen, etc. These are trees using considerable water, having a rapid growth rate, and are found usually in river bottoms and moist meadows. The slow growing trees of dry habitat do not so well withstand the ravages of smoke. The conifers mostly grown in cities of this latitude stand at the extreme of this class, that is, they are xerophytic. Yew and cypress are the more resistant and are also the more mesophytic. They have no stomatal wax. In general, there seems to be some relation between the natural moisture requirement of the conifer and its resistance or sensitiveness to smoke. At present, however, it can only be ventured as a suggestion.

Summary

1. The black deposit found in the stomatal pits of certain conifers, and thought to be soot, was found to be a natural product of the leaf.
2. The substance in the stomata of juniper, fir, and Austrian pine was found to be a wax.
3. The wax is finely granular, permeable to gases, but must greatly retard gas exchange and thus retard metabolism and water loss.

4. There is a remarkable parallel between presence of the wax and xerophytism in the conifers, and between absence of the wax and mesophytism (and hygrophytism).

5. No relation is seen to exist between wax in the stomata and the high sensitiveness of certain conifers to smoke, except that as a factor inducing xerophytism the wax may lower the resistance of the tree.

The appreciative interest and helpful criticism of Professor HENRY C. COWLES are responsible for the assembling of this material for publication. Dr. GEO. D. FULLER very kindly furnished material and information.

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BRIEFER ARTICLES

VARIATION IN VIGOR OF SPROUTS FROM QUARTERS OF SINGLE TUBERS^{*}

(WITH ONE FIGURE)

In the course of a physiological study of the potato plant, an attempt was made to secure four uniform groups of plants for four distinct treatments by quartering seed tubers. Thus each tuber would produce four plants, or groups of plants, and the plants under one treatment would be from the same original tubers as those under any other treatment. To keep all conditions as uniform as possible in starting the plants, Early Ohio tubers from cold storage were quartered along the longitudinal axis. Each seed piece was trimmed along a cut face until it weighed between 25 and 30 gm. (approximately 1 ounce). Each piece was then planted in a pot in ordinary soil that had been thoroughly mixed. They were carefully planted in the same position, each piece being placed horizontally, about two inches deep, with the uncut surface up. Thirty tubers were thus quartered and planted. As the plants were not to be subjected to the different treatments until they were of a measurable height, the pots were labeled and placed together under uniform greenhouse conditions.

In spite of the precautions in cutting and planting the seed piece, there was a marked variation in the time of appearance above the soil of the sprouts from the four pieces of the same tuber. There was an equally striking variation in the vigor of sprouts; the more vigorous sprouts in general being first to appear. As the tubers were relatively dormant when planted (early in April), most of the seed pieces produced only a single sprout. The weak, slow growing sprouts were an exception to this, for they were soon followed by still smaller secondary sprouts.

The variation was so consistent throughout the entire experimental planting, that the plants from six original tubers were examined at this stage. The seed pieces were found free from rots or other injuries. The young plants were vigorous and free from diseases, insects, or the effects of soil deficiencies. Typical plants from two quarters of the

^{*} Published with the approval of the director as Paper 432 of the Journal Series of the Minnesota Agricultural Experiment Station.

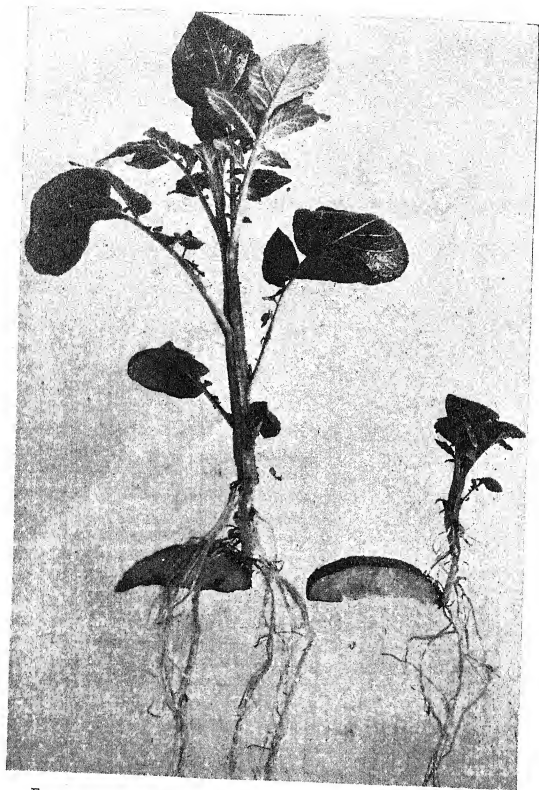


FIG. 1.—Variation due to position of sprout on seed piece; part of seed pieces cut away to show point of attachment.

same tuber are shown in fig. 1. It may be noted that the sprouts have arisen from eyes near the bud end, a condition which held in all those examined. As the seed pieces tapered toward the bud end, some of the sprouts were attached at the extreme tip. In all these cases the plants were very small. This condition was found to hold generally; that is, all of the small plants were attached at the tip or near the edge of the seed pieces, while the larger plants had arisen from more centrally located buds. A part of the seed piece has been cut away to better illustrate this. The vigor of the sprout was directly correlated with its position on the seed piece. Plants arising near the edge or at the tip apparently were unable to draw upon the reserve foods of the tuber as successfully as sprouts more favorably located.

The remainder of the experimental plants were divided into four lots according to the original plan. In each lot there was a wide variation in size of plant. Frequent measurements were made on these plants up to the period of tuberization. The stronger sprouts consistently produced the larger plants. As the experiment was discontinued at the time of tuber setting, there is no direct evidence from these data that the more vigorous plants would have produced the larger yield. STEWART,² however, has presented some evidence on this point. He measured the height of plants in the field when ten inches or less in height, and found an excellent correlation between height of plant at this stage and final yield. From this evidence it seems safe to conclude that the effect of the sprout's position on the seed piece is carried through the growing season, and to a degree affects the yield.

In an earlier paper STEWART³ reported in detail variations from halves of the same tuber of the same magnitude as the variations found in the present work. He was unable to account for the differences he observed. As the variations between hills from halves of the same tuber were entirely comparable with the variations from the quarters found here, they may have been due largely to the position of the sprouts on the seed piece. This conclusion, however, perhaps is not entirely justified, for STEWART was dealing with seed pieces that produced four to six sprouts. It would seem that several sprouts would draw more uniformly on the food reserves of the seed piece than single sprouts, and

² STEWART, F. C., Potato seed experiments: whole small tubers vs. pieces of large tubers of the same plant. New York Agric. Exp. Sta. Bull. 491. 1922.

³ ———, Further studies on the effect of missing hills in potato fields and on the variation in the yield of potato plants from halves of the same seed tuber. New York Agric. Exp. Sta. Bull. 489. 1921.

the variations between such groups of sprouts due to position on the seed piece could hardly be as great as in the single sprouts of the present study.—JOHN BUSHNELL, *Ohio Agricultural Experiment Station, Wooster, Ohio.*

THE SMEAR METHOD FOR PLANT CYTOLOGY

Cytological students of the maturation divisions in animals for some time have enjoyed the advantages of the "smear method" of preparation of tissues, by which the material is spread in a thin film on a cover glass, fixed, stained, and mounted *in situ*. (The resistant united cell walls of plant tissues would seem to preclude the use of this technique in botanical practice, but it is obvious that this condition is absent during all but the earliest stages in pollen development, which perhaps of all plant phases has received, yet still needs, the most critical study. The only botanical adaptation of the "smear method" of which the writer is aware is the use of the aceto-carmin method, especially as advocated by BELLING, a method of undoubted value where a prompt report on the progress of the main meiotic phases or on the chromosome number is all that is required. This method produces a comparatively fugitive product, however, and the mounting medium (acetic acid) is of a refractive index low enough to introduce difficulties in the precise use of oil immersion objectives. In the writer's experience the method has not been favorable for a study of spireme conditions, and even in metaphase and anaphase has caused some distortion and swelling of the chromosomes.

The "smear method," however, may be so conducted that the product can be mounted in balsam and kept indefinitely. The fixing fluid used is a chrom-osmic-acetic mixture of the following constitution: 10 per cent acetic acid 2 cc.; 10 per cent chromic acid 0.2 cc.; osmic acid dissolved to 2 per cent strength in 2 per cent chromic acid 1.5 cc.; distilled water 8.3 cc. About 1 per cent of maltose has usually been added to this fluid, which will probably have to be modified to suit each plant on which it is used. A slender glass rod is laid in the bottom of a Petri dish, and enough of the fixing fluid poured in to cover it. It has been found more convenient to use slides than cover glasses, and these are cleaned by long immersion in battery fluid, rinsed, and dried with an absolutely clean cloth. The anthers are excised, and as soon as collected are crushed and spread over the center of the slide with quick strokes of a clean scalpel, which must be honed flat and smooth on the face or

the smear will not be a success. The slide must be immediately inverted on the fixing fluid, bringing it down in a horizontal position, so that the whole smeared face is wet simultaneously. The time from the first crushing of the anther to fixing should not exceed 3-5 seconds. If the slide is brought down on the fluid in an oblique position much of the material will wash off. The slide may stay in the Petri dish, resting on the glass rod, for a few moments, while a second is prepared and placed beside it; then it may be removed, turned right side up, flooded with the fluid, and left for the full time of fixing, or about 15 minutes. The slides are then to be washed in changes of water in staining wells for about an hour, and any large pieces of anther walls, filaments, etc., which have not broken loose, can be picked off with a needle. A preliminary inspection of the material can also be made, and slides showing stages which are not desired may be discarded. The developing pollen mother cells adhere excellently, and rough rinsing does not dislodge them. Smears of pollen grains are quite easily made, so long as they are still surrounded by viscous fluid in the anther, and a very fair fixation may be secured through the chitinous wall, so that the first mitosis within the pollen grain may be critically studied.

If the slides are to be stained at once, they are then bleached in diluted aqueous hydrogen peroxide, rinsed again, and placed in 2 per cent iron ammonia alum solution; or they may be partly dehydrated and held in 70 per cent alcohol for later staining. After 4-12 hours in the iron alum they are again washed most carefully for 15 minutes or more in running water, and stained for 4-12 hours in 0.5 per cent aqueous haematoxylin solution, followed by another rinsing, destaining under observation in the iron alum, and an hour of final washing. Dehydration is to be accomplished gradually through alcohols by 10 per cent stages, but the slides need remain in each stage only 2-3 minutes. Clearing must be done by mixtures of xylol and absolute alcohol, of which about four intermediate stages seem to be enough. In this connection it must be noted that the somewhat mucilaginous walls of the developing pollen mother cells are intact, and as a result any great change in the density of the successive solutions into which the slides are introduced is sure to cause collapse of the walls and shrinkage of the protoplast. After the smears have come from pure xylol, very dilute xylol balsam is dropped on them and allowed to concentrate by drying for a few minutes before the cover glasses are put in place.

The chief difficulty encountered is that of getting a brilliant stain, but the writer has eventually succeeded in doing so in those plants

which he has so far tried. The haematoxylin and iron alum must both be of the best grade and in good condition, but in spite of care some batches of slides will show a muddy stain. Passable results can be secured within a single working day by shortening the staining periods, thus enabling chromosome count work to be done very rapidly, with the advantage over the aceto-carmin method of permanency of the slide records. Because the cells are spread out in a single layer and in immediate contact with the fixing fluid, the quality of the fixation may be very high. The karyolymph is precipitated in a uniform and delicate way, giving the much desired "solid fixation" of the nucleus, as a result of which the chromatin elements retain their normal position within the membrane, resisting the tendency to clump at various stages so familiar in paraffin material. The cells further are entire, and the observer may work without the necessity of accounting for parts of one cell displaced into two or three sections. This is not always an advantage, but in making chromosome counts it is much to be desired, and this method gives entire metaphase plates without broken or cut chromosomes. It might be thought that the making of the smear would rupture the pollen mother cells, or at least displace the contents within them, but it is found that most of the cells escape damage, and in the others it shows so obviously that there is almost no possibility of confusing normal and abnormal conditions.

The writer's experience with this method has been almost entirely limited to monocotyledonous plants, as *Gasteria* and *Veltheimia*, but from random observations and the experience of his students he is confident that it will serve for many dicotyledons also. It will probably succeed best when there is a large amount of archesporial tissue and a minimum of reserve food in the developing pollen mother cell.—WM. RANDOLPH TAYLOR, *University of Pennsylvania*.

THE HOLDFAST OF CHAETOMORPHA TORTA

(WITH ONE FIGURE)

Chaetomorpha torta (Farlow) McClatchie, one of the largest members of the genus, is frequently washed ashore on the coast of southern California; it is usually found as curled, tangled masses conspicuous by the peculiarly glassy appearance of the dark green filaments. During the period of January to May, 1923, the writer examined hundreds of these

tangled filaments without noting a specimen with holdfast intact. SETCHELL and GARDNER⁴ have written in this connection as follows:

In searching for any trace of an attachment, our persistence was rewarded by the discovery of one among all the material examined, and that, curiously enough, was found on no. 211 of FARLOW, ANDERSON, and EATON's *Algae Americae Borealis Exsiccatae*. The rhizoids are slender, blunt, somewhat branched, and evidently were attached to a rock surface.

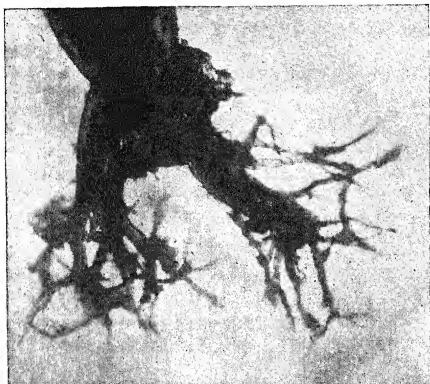


FIG. 1.—Holdfast of *Chaetomorpha tortu* (Farlow) McClatchie; $\times 40$

On February 2, 1923, while searching for lobsters under partially submerged shelving rocks a short distance north of the Scripps Institution for Biological Research, the writer felt a mass of slippery filaments attached to the under surface of a flat rock. They were typical specimens of *C. tortu*, except that the filaments were almost straight while attached to the rock. Upon being removed from the rock they immediately became coiled and twisted, after the manner of the masses found along the shore. A number of specimens were carefully removed from the rocks with little injury to the holdfasts. One of these, shown in fig. 1,

⁴ SETCHELL, W. A., and GARDNER, N. L., The marine algae of the Pacific Coast of North America. Part II. Chlorophyceae. Univ. Calif. Publ. Botany 8:199-206. 1920.

was photographed at once. The holdfasts withered quickly and were not easily studied on the specimens after drying. It will be noted from the figure that the rhizoids are relatively large where they branch from the main filament, and that they are considerably branched. These points are in contrast with the description by SETCHELL and GARDNER; the fresh material undoubtedly permitted of more satisfactory examination than did the one herbarium specimen mentioned in their description.

In discussing the habits of *C. torta* and *C. spiralis* Okam., SETCHELL and GARDNER use the terms "erect and tufted" for one group of members of the genus, and the terms "prostrate or flexuous or crisped habit, intertwining and becoming detached" for another group. The writer suggests that the habit of *C. torta* might be described as pendent in locations such as that described in this article. It seems possible that the curled and contorted habit of detached specimens may be due to the release of tensions which serve to keep the filament almost straight while it is attached to the rock.

The writer is indebted to Dr. N. L. GARDNER for confirming the identification of *C. torta*.—ORVILLE TURNER WILSON, *University of Cincinnati*.

CURRENT LITERATURE

BOOK REVIEWS

Beneke-Jost Plant Physiology

The exhaustion of the third edition of JOST's *Lectures on Plant Physiology*, which appeared about ten years ago, has led to the revision of the text by the author, with the collaboration of BENEKE. The new fourth edition by BENEKE and JOST¹ is published in two volumes, the first of which, revised by BENEKE, deals with metabolism, and the second volume, prepared by JOST, covers the field of growth and movement.

There has been no change in organization of the first volume, which contains nineteen chapters with the same chapter headings as the first nineteen lectures of the previous edition. The second volume, however, shows some notable changes in organization of contents. The eleven lectures of part II of the former edition have been replaced by three chapters, the first of which introduces the problems of growth of cells and organs, and methods of measuring growth. The second chapter deals with the external and internal factors which cause and modify or condition the rate of growth. The third chapter considers the whole developmental process of plants, from the resting seed or bud to senescence and death. The twelve lectures on movement, part III of the older edition, are replaced by four chapters, dealing with hygroscopic bursting and throwing movements, tropisms, nasties, and locomotor movements, or taxies. These changes of organization in the second volume make a decided improvement from the standpoint of mere organization.

The revision seems to the reviewer to be fairly adequate from the European point of view, their literature having been well summarized. The American plant physiologists, however, will find it necessary to summarize and incorporate the advances made here mainly from the original sources. As the senior author remarks: "So lege ich auch ohne Gefühl der Befriedigung die Feder nieder in dem Bewusstsein dass vieles besser hätte werden können." Difficulties have no doubt lain in the way of a thorough analysis of our contributions to plant physiology; but this must not continue to be an excuse for failure to internationalize the results of scientific investigation. This new edition will be desired by every student of plant physiology, regardless of the obvious shortcomings with reference to American literature.—C. A. SHULL.

¹ BENEKE, WILHELM, and JOST, LUDWIG, *Pflanzenphysiologie*. Two vols. 8vo. viii+441; and viii+477. Jena. Fischer. 1923.

Standardized plant names

A volume under this name has just been published,² which is the result of the cooperative efforts of more than a dozen of the leading horticultural societies of America, and has for its object the adoption of a uniform nomenclature of the scientific and common names of plants cultivated in the United States. The scientific names approved are almost entirely in accord with the international rules of nomenclature. In standardizing the common names, it has been found necessary not only to select from those already in use, but also to bestow a number of new names, and even then some plants are without any common designation. For these the public are invited to make suggestions. The adopted names are declared standard for not less than a five-year period.

The purpose of the work is a most commendable one, and on the whole the results seem to be excellent. The volume is convenient, both in size and in the alphabetical arrangement of its contents, with different faced types for scientific, common, and synonymous designations. Plants with recognized Latin and common names appear in the list under each name. A large number of horticultural varieties is included.—GEO. D. FULLER.

Micro-organisms of soil

RUSSELL and co-workers³ have published an important book dealing with the micro-organisms of the soil. It is one of the Rothamsted monographs. The organisms included are bacteria, protozoa, algae, fungi, and invertebrate fauna (other than protozoa). Each group is dealt with by the person in charge of the particular department at Rothamsted. In a beginning chapter RUSSELL develops the idea of a soil population, considering the historical features and furnishing the background for the later chapters. In a final chapter he considers the chemical activities of these organisms, and relates them to the growing plant. The book constitutes a good critical summary of what we know concerning the micro-organisms of the soil. At the same time, each chapter is teeming with the many problems that remain to be solved in this important and almost virgin field of investigation. RUSSELL and his associates have done a real service to the general biologist as well as to the specialist in plant physiology and agriculture in publishing this book.—S. V. EATON.

A textbook on botany

SINNOTT⁴ has published an attractive textbook of botany which is the outgrowth of teaching college freshmen. The mechanical execution is excellent,

² OLMSTEAD, F. L., COVILLE, F. V., and KELSEY, H. P., *Standardized plant names: a catalogue of approved scientific and common names of plants in American commerce*. 8vo. xvi+546. Salem, Mass. Amer. Joint Comm. on Horticultural Nomenclature. 1924.

³ RUSSELL, SIR E. JOHN, and others, *The micro-organisms of the soil*, pp. vii+188, figs. 24. Longmans, Green & Co. 1923.

⁴ SINNOTT, E. W., *Botany, principles and problems*. Pp. xix+385, New York: McGraw Hill Book Co. 1923.

the figures clear and to the point. The style is lucid, and the subject matter well selected. After an introductory survey, the author discusses soil, the structure and function of root, leaf, and stem. Metabolism and growth are the next chapter topics; then the plant, its environment, reproduction, heredity, variation, and evolution. The last five chapters are devoted to a survey of the plant kingdom. At the conclusion of each chapter there is a series of questions for thought and discussion, 816 questions in all, and also a list of 144 problems. The latter require the study of material outside of the book; the former may be answered from the information the book imparts. These questions and problems should serve as a stimulating help to the teacher.—E. R. DOWNING.

NOTES FOR STUDENTS

Taxonomic notes.—DOMIN, of Prague, has published a long delayed paper on additions to the flora of Western Australia. It was presented to a scientific society in 1917, but publication was much delayed by "difficulties involved in publication during the war and after it." The publication (1923) of 123 pages includes numerous species, many of which are new, and among them a new genus (*Nemcia*) of Compositae.

MISS OTTLEY⁵ has published a very complete and well illustrated revision of the Californian species of *Lotus*, recognizing 29 species. It is interesting to note that 24 of these species are referred to GREENE, and also that the genus *Hosackia* is included in *Lotus*.

RIDLEY,⁶ in preparing the Euphorbiaceae for the *Flora of the Malay Peninsula*, has discovered 21 new species, representing 14 genera, one of the genera (*Actephilopsis*) being new.

WILSON⁷ has published a revision of *Dicoma* (Compositae), a genus of tropical and southern Africa and the East Indies, recognizing 34 species, only one of which is described as new. The fact that this genus is credited with only 13 species in DURAND's *Index Generum Phanerogamorum* of 1888 illustrates the development of knowledge of the African flora.

BRACKETT⁸ has published a revision of the American species of *Hypoxis*, recognizing 15 species, 4 of which are described as new. This genus occurs mostly in the southern hemisphere.

PEARSON⁹ has published an account of collections of New Zealand Hepaticae, made by SETCHELL in 1904, which includes descriptions of 15 new species distributed among 12 genera.

⁵ OTTLEY, ALICE M., A revision of the Californian species of *Lotus*. Univ. Calif. Publ. Bot. 10:189-305. pls. 61-82. maps 10. 1923.

⁶ RIDLEY, H. N., New Euphorbiaceae from the Malay Peninsula. Bull. Kew Gardens. no. 10. 366-369. 1923.

⁷ WILSON, F. C., Revision of the genus *Dicoma*. Bull. Kew Gardens. no. 10. 377-388. 1923.

⁸ BRACKETT, A., Contrib. Gray Herb. N.S. 69. pp. 120-163. figs. 17. 1923.

⁹ PEARSON, W. H., Univ. Calif. Publ. Bot. 10:307-392. pls. 83-109. 1923.

JOHNSTON¹⁰ has published a second paper on Boraginaceae, which is a synopsis of the American native and immigrant species of the subfamily Boraginoideae. It includes 31 genera, one of which (*Lasiarrhenum*) is new. The largest genus is *Lithospermum* with 32 species, 4 of which are new. There are numerous new varieties and combinations. There is also given a tentative classification of the South American species of *Coldenia*, 8 species being recognized, and a new section (*Sphaerocarya*) proposed. In the same contribution some "taxonomic records" are published, including 27 new species in various genera.

MUNZ¹¹ has published a revision of *Nemacladus*, chiefly a Californian genus, assigned by TORREY to the Lobeliaceae, but now included in the Campanulaceae. MUNZ recognizes three species, *N. longiflorus*, *N. ramosissimus* (with two varieties), and *N. rigidus* (with five varieties).

ROBINSON,¹² in continuing his studies of the tropical American Eupatorieae, has described 35 new species, 27 of which belong to *Eupatorium*.

JOHNSTON¹³ has described new genera of Euphorbiaceae (*Halliophytum*) and Compositae (*Eremonanus*) from California, in addition to numerous new species.

RYDBERG,¹⁴ in his investigation of the genera of North American Fabaceae, has begun the publication of his results in reference to the tribe Galegeae. He recognizes 11 subtribes, 5 of which are presented in the present contribution, including 12 genera, one of which (*Daubentoniopsis*) is described as new, based on *Aeschynomene longifolia* Cav.—J. M. C.

Temperature and flowering.—A study of the blooming habits of *Carnegiea gigantea* has been made by JOHNSON,¹⁵ who finds that the flowers on the east side of the crowns develop more rapidly and open earlier than those situated on other parts of the crown, and that they are more numerous on the east half of the plant. The various factors which might cause these differences in distribution and rate of development are considered, with the conclusion that temperature differences in the tissues of the crown are responsible for the observed facts. The early insolation of the east side of the plant brings about a heating effect which produces a temperature about 2° C. higher in the tissues

¹⁰ JOHNSTON, I. M., Contrib. Gray Herb. N.S. 70. pp. 1-92. 1924.

¹¹ MUNZ, P. A., A revision of the genus *Nemacladus* (Campanulaceae). Amer. Jour. Bot. 11:233-248. 1924.

¹² ROBINSON, B. L., Records preliminary to a general treatment of the Eupatorieae. III. Contrib. Gray Herb. no. 68. 1-43. 1923.

¹³ JOHNSTON, I. M., Diagnoses and notes relating to the Spermatophytes chiefly of North America. Contrib. Gray Herb. no. 68. 80-104. 1923.

¹⁴ RYDBERG, P. A., Genera of North American Fabaceae. I. Tribe Galegeae. Amer. Jour. Bot. 10:485-498. 1923.

¹⁵ JOHNSON, D. S., The influence of insolation on the distribution and on the developmental sequence of the flowers of the giant cactus of Arizona. Ecology 5:70-82. 1924.

of that side. The temperature difference reaches a maximum at about ten or eleven o'clock, but persists as actually higher in temperature than other parts of the crown until about three in the afternoon. After that time the west side may be warmer than the other parts, but its period of high temperature is much briefer than that of the east side. This higher average temperature of the eastern and southeastern parts of the crown is believed to be nearer the optimum temperature for growth of the cactus buds and flowers, and more favorable to a high percentage of bud development.

Evidence is accumulating to show that temperature is a very potent influence in the blooming behavior of plants, and that photoperiodic responses may be very greatly modified by temperature effects. This is probably associated with the internal nutritive conditions of the tissues, flowering depending upon some sort of balance of nutritive relations which is upset by the independent variation of the factors which determine those nutritive conditions, mainly light and temperature.—C. A. SHULL.

Permeability.—In a series of papers, just concluded, STILES¹⁶ has summarized the results of workers in the field of permeability, indicating in a critical manner the chief problems. A discussion of the complexity of the system involved, and the inadequacy of our knowledge of it, serve as an introduction. It is believed that the cell wall is a part of that system. Physico-chemical relations are discussed under such chapter headings as Surface phenomena, Diffusion, Permeability of membranes, Osmotic pressure. In a consideration of the internal conditions which influence the passage of water through the cell wall and protoplasm, the simple osmotic view of the cell is emphasized and its inadequacy shown. Particular emphasis is laid on exact measurements of the effects of temperature, and the composition of the external medium. The chief problem in understanding the passage of dissolved salts is the perfection of quantitative methods for determining intake and outgo. Few reliable data are available. The author feels that no theory of permeability has sufficient quantitative data to support it as yet, and the real usefulness of theories is stimulative. The main problem in the field of permeability is to get sufficient quantitative data that the laws governing the intake of substances from the external surroundings, passage outward from the cell, and translocation from cell to cell within the organism may be formulated. A complete bibliography is included. These papers are so valuable that they should be made available to physiologists in the form of a reprint.—W. B. DAVIS.

Phosphates in relation to algal plankton.—Using colorimetric methods, ATKINS¹⁷ has studied the phosphate content of various salt and fresh waters. He

¹⁶ STILES, W., Permeability. *New Phytol.* 20:45-55, 93-106, 137-149, 185-194. 1921; 21:1-14, 49-65, 140-162, 169-209, 233-251. 1922; 22:1-29, 72-94, 128-149, 204-224, 239-280. 1923.

¹⁷ ATKINS, W. R. G., The phosphate content of fresh and salt waters in its relationship to the growth of the algal plankton. *Jour. Marine Biol. Assn.* 13:119-150. 1923.

finds that the phosphate content of salt waters decreases from March to July, when it may be zero. In the case of certain fresh water ponds, the amount of phosphorus present is almost zero by April, and continues low throughout the summer. To explain this decrease in the phosphorus content, the hypothesis is advanced that it is due to an increase in algal plankton, the algae using the phosphorus in the building of body tissues. In support of this hypothesis, it was found that in the case of pure laboratory cultures of diatoms, the phosphate content decreased as the number of diatoms increased. Also, sea water insulated in the laboratory showed a gradually decreasing amount of phosphorus present. Data as to the amount of phosphorus used by diatoms and the phosphate content of sea water are used to estimate the theoretically possible algal plankton crop, and also the possible fish production. Many algae are eaten by animals, and so the actual number of algae present is always far below the possible number. ATKINS' work shows an interesting correlation between the phosphorus content of fresh and salt waters and the number of algae present.—S. V. EATON.

Effect of hydroxyl ion on growth.—There have been many contributions in recent years on the effect of the hydrogen ion on growth, and the field has been somewhat overworked. Not so much attention has been given to the hydroxyl ion. REED and HAAS¹⁸ report the results of experiments concerning the effect of the hydroxyl ion on the growth of walnut roots. They consider the question as to whether the sensitivity of walnuts to alkaline conditions is due to the hydroxyl ion or to the lack of calcium. Their results indicate that the latter is the case. The roots were quickly injured in alkaline nutrient solutions from which calcium was omitted. On the other hand, the roots remained healthy in a calcium hydrate solution of P_H 9.0 or higher. Roots injured in alkaline calcium-free nutrient solutions quickly recovered when transferred to solutions containing calcium, while healthy roots transferred from a calcium hydrate solution to an alkaline solution lacking calcium were soon injured. Such experiments indicate that the bad effects of alkaline conditions on walnut roots are not due to the hydroxyl ion as such, but to the fact that in these alkaline conditions the calcium is precipitated from solution. The paper is well illustrated by text figures.—S. V. EATON.

Vegetation of the Carpathians.—In a recent publication, DOMIN¹⁹ has outlined the zonation of vegetation in the Western Carpathians, which in general rise from a lowland of oak or mixed deciduous forest. Above this the usual zonation is: (1) a submontane zone with the upward extension of the lowland

¹⁸ REED, H. S., and HAAS, A. R. C., The effect of the hydroxyl ion concentration on the growth of walnut roots. *Amer. Jour. Bot.* 11:78-84. 1924.

¹⁹ DOMIN, K., A phytogeographical outline of the zonal division of the Western Carpathians, besides some general remarks on the main forest trees. *Publ. Faculte des Sciences Univ. Charles. Prague.* pp. 43. 1923.

forest with some admixture of beech, fir, and pine; (2) a montane beech zone; (3) an upper montane spruce zone with an upper limit ranging from 1350 to 1500 m.; (4) a subalpine zone of dwarf pine (*Pinus montana*) up to 1500 to 2000 m.; and (5) an alpine zone. Not the least interesting and important part of the study consists of an analysis of the factors which cause variations in the limits of these zones or even their inversion. For example, in deep valleys the spruce may occupy the damp, cool, lower portions, and be surmounted on the drier sunnier slopes by a beech zone, or either the beech or the spruce may occupy both the lower and the upper montane zones. Emphasis also is given to the variations in the altitudinal limits of the zones due to soil, exposure, and other agencies.—GEO. D. FULLER.

North American flora.—The fourth part of volume 25 contains the completion of Geraniales, the Meliaceae by PERCY WILSON and the Trigonaceae by P. C. STANDLEY; and also the beginning of Polygalales, the Vochyaceae by P. C. STANDLEY and the first instalment of Polygalaceae by D. F. BLAKE. In Meliaceae 7 genera are recognized, including 88 species, the large genera being *Gaurea* and *Trichilia*, each with 33 species. The family Trigonaceae is represented by a single genus with 2 species, and Vochyaceae includes a single genus with 4 species. The family Polygalaceae is begun with *Polygala*, in which 179 species are recognized, 43 being presented in the present part, 3 of which are described as new.—J. M. C.

Anatomy of Selaginella.—Miss STEEL²⁰ has published an interesting account of the anatomy of *Selaginella uliginosa*, a common species of Eastern Australia. The shoot has the radial type of vascular cylinder, which is now recognized as the most primitive type of stem cylinder, persistent chiefly in roots. The rhizome is solenostelic, and the root protostelic, with only one group of protoxylem. The general conclusion is that *S. uliginosa* is one of the more primitive species of the genus. The combination of heterospory with a primitive vascular situation is a combination which makes *Selaginella* an unusually interesting type.—J. M. C.

The strobilus theory.—PARKIN²¹ has restated and amplified the theory of the origin of angiosperms proposed by the late Dr. NEWELL ARBER and himself in 1907. He assembles evidence to show that Ranales contain the families with least modified flowers (really strobili), from which all other flowers have been derived by reduction and modification. This discards the Engler series in regard to Amentiferae as the most primitive. The Ranales, in turn, are regarded as related in origin to such Gymnosperms as the Bennettitales, at least in common origin from the "Pteridosperms." The details of data and conclusions are interesting, although not always convincing.—J. M. C.

²⁰ STEEL, JESSIE K., Anatomical features of the mature sporophyte of *Selaginella uliginosa*. Proc. Linn. Soc. New South Wales 48:287-300. 1923.

²¹ PARKIN, J., The strobilus theory of angiospermous descent. Proc. Linn. Soc. London. pp. 51-64. 1923.

Hydrogen ion determination of tissues.—A method of determining the hydrogen ion concentration of tissues colorometrically is described by ATKINS,²² who applies the indicators directly to sections or to the crushed tissues. Attention is called to the usefulness of diethyl red in regions of acidity where methyl red and brom cresol purple are yellowish, and hard to distinguish from tissue colors when applied directly. Many tissues lie in this range. It is also pointed out that methyl orange is useful in more acid regions where brom phenol blue is yellow.—W. B. DAVIS.

Evolution of herbaceous angiosperms.—SINNOTT and BAILEY²³ have felt the necessity of restating their conclusions in reference to the evolution of herbaceous angiosperms from arborescent or fruticose ancestors, published in 1914.²⁴ These conclusions have been criticized since that time in such a way that the authors have endeavored "to clarify the real points at issue," and thus correct what seems to be a misunderstanding.—J. M. C.

Embryo sac of *Oenothera rubrinervis*.—O'NEAL²⁵ has investigated the development of the embryo sac of *Oenothera rubrinervis*, and in addition to the usual events records the following facts: the micropylar megaspore usually becomes the functional one; the mature sac contains but four nuclei; fertilization occurs about thirty-six hours after pollination and is probably "single."—J. M. C.

Embryogeny.—SOUÈGES,²⁶ in continuation of his numerous studies of embryogeny, has published the usual very detailed and well illustrated accounts of embryo development in *Myosotis hispida* and *Geum urbanum*.—J. M. C.

²² ATKINS, W. R. G., The hydrogen ion concentration of plant cells. Notes Bot. School Trinity College, Dublin 3:178-198. 1922.

²³ SINNOTT, E. W., and BAILEY, I. W., The significance of the "foliar ray" in the evolution of herbaceous angiosperms. Ann. Botany 36:523-533. pls. 18, 19. fig. 1. 1922.

²⁴ Review in Bot. Gaz. 60:247. 1915.

²⁵ O'NEAL, C. E., A study of the embryo sac development and accompanying phenomena in *Oenothera rubrinervis*. Bull. Torr. Bot. Club 50:133-146. pls. 5, 6. 1923.

²⁶ SOUÈGES, R., Développement de l'embryon chez le *Myosotis hispida* Schlecht. Bull. Soc. Bot. France. Ser. IV. 23:385-401. 1923.

———, Développement de l'embryon chez le *Geum urbanum* L. Ibid. 64:5-66. 1923.

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DOUBLED CHROMOSOMES OF OENOTHERA LAMARCKIANA SEMIGIGAS

HUGO DE VRIES AND K. BOEDYN

(WITH TWO FIGURES)

Introduction

In a previous paper (8) we have tried to show that the numerous types of the mutants of *Oenothera Lamarckiana* may be combined into groups. We started from two principles: the frequently repeated production of one type by another, and the striking external resemblance of forms which were different in their hereditary features. *O. mut. albida* is produced almost yearly by *O. mut. lata*, and in a relatively high percentage. *O. mut. oblonga* almost always is seen in large numbers among the progeny of *O. scintillans*. It is evident that such repeated productions point to some kind of intimate connection, such as can be explained only on the assumption that the factors for the two types are located in the same chromosome. On the other hand, in all its organs *O. mut. nitens* shows the same characters as *O. mut. scintillans*, although the first type is constant, whereas the other splits, in each generation, into specimens of its own form and others of the type of *O. Lamarckiana*. The external features being the same for both, their factors must also be assumed to be the same, at least mainly. *O. mut. nitens* and *O. mut. scintillans*, therefore, were assumed to be located in the same chromosome.

Applying these principles to the great majority of the mutants, we had seven groups, corresponding to the seven chromosomes of the haploid nucleus. One of them consisted chiefly of the isogamic mutants, but in each of the others one of the dimorphic or splitting mutants prevailed. Accordingly we proposed to call the chromosomes after these. The name of central chromosome was chosen for the one containing mainly isogamic types, and two of the others were called after *O. mut. lata* and *O. mut. scintillans*, reserving the names of *cana*, *pallescens*, *liquida*, and *spathulata* for the four remaining ones. The three first groups were found to be large; they may correspond to the three large chromosomes described and figured by CLELAND for *O. franciscana* (2). The four other groups would then correspond to the four smaller chromosomes of the haploid nucleus. We have since found these small and large chromosomes in the nucleus of *O. Lamarckiana* itself.

It is well known that the heterogamic mutations of *O. Lamarckiana* as a rule are connected with the doubling of one of the chromosomes. This brings their number in the vegetative cells up to 15. Why this should be so we do not know, since the doubling may be accompanied, for each chromosome, with widely different types of mutations, such as *albida* and *lata* in one case, or *oblonga* and *scintillans* in another. Moreover, the hereditary features are not determined by this doubling, since in almost every group *sesquiple*x or constant forms are found besides the typical dimorphic or splitting ones. The connection is such, however, that in ordinary cases one may confidently rely upon it for conclusions.

It would follow that the progeny of mutants of the type of *semigigas* might afford a good test for our conceptions. It is astonishingly rich in forms, some of which repeat the old types (*O. lata*, *O. scintillans*, and *O. oblonga*) in a relatively high percentage, whereas others constitute new types occurring in smaller numbers or even in single individuals, as has been described by one of us (4). At that time (1913), however, only three of the dimorphic prototypes (*O. lata*, *O. scintillans*, and *O. spathulata*) had been discovered, and thus the principle for grouping the numerous forms failed. *O. cana*, *O. pallescens*, and *O. liquida* have since been distinguished (5), completing the series. If we

apply these forms as models for distinction to the motley progeny of *semigigas*, the main groups at once become prominent. Besides *lata* and *scintillans*, which are always easily recognized, *cana* and *spatulata* become evident, and *palescens* and *liquida* can also be distinguished, although not with the same clearness. In some specimens these types seemed to be as pure as those of the corresponding old races, but in many others they were evidently obscured by subordinate deviating characters.

From numerous cultures of the progeny of different types of *semigigas*, we discovered that the clearness of the groups depends largely on the choice of the type which yields the pollen for the fertilization. In order to get a sufficient supply of good seeds the flowers of *semigigas* have to be fecundated by means of other pollen; self-fertilization produces too few germinating seeds. Some pollen proved to be dominant over a majority of the mutations, making the distinction very difficult and in some degree uncertain (as that of *O. Lamarckiana* mut. *gigas*); other types proved to be recessive, allowing a clear grouping. Among these, the forms of *velutina* were the most reliable.

For this reason we have chosen for the present investigation the pollen of the twin hybrid *O. (biennis* × *Lamarckiana*) *velutina*. There are many mutants, especially among the *sesquiple* types, which yield sufficiently pure *velutina* pollen (6), and some of them have been used for control experiments, as will be shown elsewhere. The twin hybrids of *O. Lamarckiana* are old forms described since 1907, however, and studied by many investigators. Moreover, they easily may be reproduced by anyone interested in the present discussion.

OENOTHERA (LATA × LAMARCKIANA) SEMIGIGAS

In 1915 a mutant *lata* originated from the pure race of *O. Lamarckiana*, and was chosen for the production of a new line. Every generation of this line has been fertilized by the pollen of *O. Lamarckiana*, since the *lata* plants had no good pollen, as is usual in our climate. In the third generation, in 1921, two rosettes of the type of *semigigas* were observed. The chromosomes in the root-tips of both of them were counted and found to be 21. The

plants remained in the condition of rosettes of leaves until the winter, and flowered in 1922, when they were used for this experiment. They were cultivated in the glass covered part of the experiment garden at Lunteren, under very favorable conditions, and flowered richly. On one of them thirty flowers of the side branches were pollinated, after a castration, with the pollen of *O. (biennis* \times *Lamarckiana*) *velutina*. They yielded 30 apparently good fruits, with 3.3 cc. of seeds, about one-quarter of which contained viable germs, as was proved in a test-tube at 30° C. The other seeds had imperfect germs of different sizes.

The harvest was sown at the end of January, 1923, in a glass-house, and 81 seeds germinated. The young seedlings showed a motley assembly of types of leaves, among which some were more frequent and could soon be recognized. After two months they were transplanted singly into small pots, and from these they were planted in the beds, in May and June, as soon as their root tips had been fixed for the counting of chromosomes. At that time the mutant characters could be recognized clearly in most of the rosettes of root leaves, and the plants were grouped accordingly before being planted. This method has the advantage that, at the time of flowering, the mutants belonging to the same type were side by side. Thus the different groups could easily be distinguished, and also the slight deviations in the individual characters within each group could be subjected to a careful comparative study. Most of the 81 mutants flowered during July and August, and ripened their fruits in the fall. They were stout, healthy plants, whose characters could be compared with those of the older mutant races, all of which flowered at the same time in the experimental garden.

This comparison was begun in the stage of rosettes and continued through the summer, until the ripening of the fruits. As a rule the rosettes show their type distinctly at the time of planting, or, on weaker plants, some weeks later. Then the young stems develop with a less differentiated foliage, but the spikes of flower buds are seen to develop once more the marks of the groups. Moreover, the deviations from the main types are usually small during youth, but become more pronounced with advancing age.

This article reports only the results of the cross *semigigas* × *velutina*. The reciprocal cross would not produce a similar deformity. We may conclude this from a study of the effect of the pollen of our plant in other different crosses, made in 1922, partly on other species, partly on the parent type and some of its mutants. For each of these crosses 10–15 or more flowers were pollinated, and yielded 40–60 or more viable seeds. In May the rosettes had reached the stage in which the marks of *laeta* and *velutina* are easily distinguished, but no *velutina* were found. Thereupon 12 specimens from each cross were planted out and cultivated during the whole summer, until they began to ripen their seeds. In the crosses with *O. biennis*, *O. grandiflora*, and *O. blandina* they were exactly like the *laeta* from the corresponding crosses with *Lamarckiana*. In *Lamarckiana* × *semigigas* they repeated the marks of the mother, and in *lata* × *semigigas* they were partly *lata* and partly *Lamarckiana*. No other types were observed in these cultures, which embraced in all over 400 rosettes and 60 flowering plants. The high degree of mutability, shown by the cultures derived from the egg cells of *semigigas*, failed in those from the pollen. From this experiment, therefore, we conclude that the pollen of our *semigigas* contains, among its viable gametes, only or almost only such of a sufficiently pure *laeta* type.

DOUBLING OF CHROMOSOMES

STOMPS (11, 12) was the first to introduce the study of *O. mut. semigigas* into the discussion concerning the stage at which the doubling of the chromosomes takes place. He counted its chromosomes and found them to be 21. From this he concluded that the doubling must occur in the production of the sexual cells before fecundation. The *semigigas* would then originate by the fertilization of a doubled gamete by a normal one, whereas the conjugation of two doubled ones would yield a *gigas*. This conception has since been generally accepted, although it was in flagrant contradiction with the older view of a doubling after fertilization, as had been proposed by STRASBURGER. ERNST (9) has formulated the theory of STOMPS clearly, stating that in the production of the mother cells of the pollen and the embryo sac, the reduction division is sometimes

omitted. This, of course, must be a rare phenomenon, and may be considered the consequence of some internal mutation.

On the basis of this view, the term doubling is rather a misleading one. In the process of synapsis the chromosomes conjugate, and at the time of the reduction division they quit each other. If this latter event is omitted, they must remain in the combined condition at least for some time, and thus cause the image of a doubling; and if this doubling is considered to be a phenomenon of mutation, we must assume that the separation of the paired chromosomes in reduction is determined by some special factor, which may become inactive in rare instances and from an unknown cause, thus producing the mutation. This view is a direct consequence from the theory of STOMPS, and is interesting for our present discussion, inasmuch as it throws light on the relation between the process of doubling and the external mutations which ordinarily accompany it. This relation cannot be considered a causal one, but only one of binding or linkage. In the description of our experiment we shall find some direct proofs for this conception (*O. Lamarckiana* with 15 and 20 chromosomes).

In the synapsis of *semigigas*, the doubled chromosomes are paired with the single ones, and during reduction they separate, and one of each pair goes to one pole and the other to the second pole. STRASBURGER pointed out that in this process the distribution of the chromosomes depends upon chance. One nucleus will receive a larger number of rods from the maternal group, and another more from the paternal, and many may have equal groups of both. ZIEGLER (13) then applied to this conception the laws of probability, showing that the distribution must proceed according to these. Applying this principle to the case of *semigigas*, we should expect its doubled chromosomes to be distributed among the egg cells according to chance.

This conclusion has been tested by VAN OVEREEM for the case of *O. Lamarckiana semigigas* on the individuals of this form which had arisen in the cultures of one of the writers in 1913. When this mutant is fertilized with the pollen of *O. gigas*, almost all the seeds are viable, or at least a sufficient part of them, and the numbers of the chromosomes may be expected to lie between 21 and 28, indicat-

ing 7 and 14 doubled ones. If an egg cell of *semigigas* without such is fertilized, the germ will have 7 single and 7 doubled ones, and $7+14=21$ rods will be counted in the nuclei. If, however, egg cells whose chromosomes are all doubled are considered, there will be two sets of 7 doubled ones, giving together 28 visible rods. From the numbers given by VAN OVEREEM, the numbers of double rods in *semigigas* may be calculated after this rule. He found for this cross, among 87 seedlings:

Chromosomes.....	21	22	23	24	25	26	27	28
Number of seedlings....	1	2	12	21	25	19	6	1

This gives for the number of doubled chromosomes in the egg cells the same numbers:

Doubled rods.....	0	1	2	3	4	5	6	7
Egg cells.....	1	2	12	21	25	19	6	1

The number of possible cases in this row being 8, we chose for the calculation of the chances the curve corresponding to $(a+b)^7$. The height of the ordinates of this curve is proportional to 1.7.21.35.35.21.7.1. The sum of these numbers being 128, and that of the seedlings 87, we must multiply the latter by $128/87$ in order to compare them. Assuming further that the empirical curve should be symmetrical, we calculate the means between its two halves. In this way we have:

DOUBLE CHROMOSOMES IN THE EGG CELLS OF *O. semigigas*

Chromosomes.....	0	1	2	3	4	5	6	7
Egg cells calculated.....	2	3	17	31	37	28	9	2
Eggs calculated symmetrically...	2	6	22	34	34	22	6	2
$(a+b)^7$	1	7	21	35	35	21	7	1

The coincidence is as close as could be expected, and shows that in the reduction division of *O. semigigas* the doubled chromosomes are distributed among the two daughter nuclei according to the laws of chance.

A main condition in this discussion has been that all or almost all the seeds are viable. If this is not so, the result may be different. In the case of *semigigas* \times *velutina*, only about one-quarter of the seeds were well developed, and the question arises, what influence this may have on the figures. In order to answer this, we assume that in our case the simultaneous occurrence of two or more

doubled rods in the nuclei of a seedling is detrimental for its development. The doubling is assumed to be connected with the same mutations of the normal characters with which it is connected in the old heterogamic races, and these mutations are probably more or less incompatible with one another. We may conclude this from the fact that mutations with 15 chromosomes, that is, with one doubled rod, are frequent, whereas those with 16-20 chromosomes as yet have not or almost not been discovered outside of the progeny of *semigigas*. Their chance of survival, therefore, must be very small; most of them, when produced in the germ, must be lost before the ripening of the seed.

As yet there is no reason to assume a lessened viability for plants with only one doubled rod. They must be as good, in this respect, as the normal races with 15 chromosomes. This leads us to expect a high proportion of seedlings with this number, and gradually smaller figures for those with more chromosomes.

NUMBER OF DOUBLE CHROMOSOMES AMONG PROGENY OF
OENOTHERA (LATA × LAMARCKIANA) SEMIGIGAS

Our culture embraced 81 seedlings, which were planted singly in small pots as early after germination as possible. As soon as a sufficient number of root leaves had developed, root tips were prepared, and thereupon the young plants were placed in the beds of the glass covered part of the garden, without their pots. Their development differed very much in rapidity; accordingly the fixation was performed at different times in May and June, 1923.

The fixations were made early in the morning, using BOVIN'S fluid. The preparations measured 5-10 μ , mostly 10 μ , in thickness, and were stained with the haematoxylin solution of Haidenhain. The general results of the counting were as follows:

Chromosomes.....	14	15	16	17	18	19	20
Number of plants.....	3	35	19	13	3	4	4

These numbers yield an asymmetrical curve, as is shown in fig. 1. The explanation of the asymmetrical form of the curve depends on the assumption that the chance of viability became smaller with an increasing number of double chromosomes. In this respect the result of the fertilization of *semigigas* with pollen of

velutina would differ essentially from that with pollen of *O. gigas*. It is interesting to note, therefore, that even in the living seedlings the chances are better, the fewer the number of double rods. This may be proved by comparing the degree of development, attained before the fall of the first year, with the numbers of such bodies.

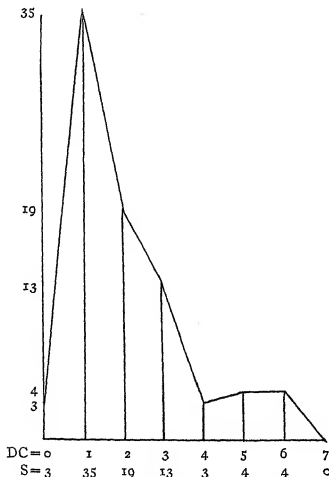


FIG. 1.—Numbers of chromosomes in seedlings of *Oenothera* (*lata* × *Lamarckiana*) *semigigas* × *O. (biennis* × *Lamarckiana*) *velutina*; DC, doubled chromosomes; S, seedlings.

Among the 81 plants, 12 remained in the stage of rosettes (one of them died before the fall, being very weak); 9 made a stem, but did not reach the flowering period; 13 flowered, but had barren anthers, at least in the beginning; and only 47 developed throughout in a normal way. If we consider these four groups as a measure of the individual strength, and compare them with the numbers of chromosomes, we get the data given in table I.

In order to get an easier survey we combine the figures into groups, and calculate their percentages (table II). This table shows that the larger part of the normal plants had 14-15 chromosomes, whereas the rosettes had mostly an abnormally high number. In other words, the development is the more incomplete, the larger the number of mutated chromosomes. From this we may safely conclude that among the sterile seeds the high chromosome numbers

TABLE I
FALL OF 1923

	Number of doubled chromosomes						
	0	1	2	3	4	5	6
Rosettes.....	0	0	4	3	3	1	1
Young stems.....	0	2	2	3	0	2	0
Barren anthers.....	0	4	6	2	0	1	0
Normal plants.....	3	29	7	5	0	0	3
Total.....	3	35	19	13	3	4	4

TABLE II
PERCENTAGES OF CHROMOSOMES

	Number of chromosomes		
	14-15	16	17-20
Rosettes.....	0	33	67
Young stems.....	22	22	56
Barren anthers.....	31	46	23
Normal plants.....	68	15	17

must prevail, leaving only a small proportion for the viable germs, and this is sufficient proof for the validity of the assumption just made.

CHROMOSOMES AND EXTERNAL FEATURES

In studying the progeny of another form of *semigigas*, one of the writers found that the offspring may be combined into groups, and that the majority of these correspond to the dimorphic mutants previously described (7). *O. (blandina × simplex) semigigas* (*O. perennis*) was fertilized, partly with pollen of other species, partly with that of different mutants of *O. Lamarckiana*, and the progeny

counted at the time of flowering. Six main groups were distinguished, corresponding to the old dimorphic races, and among these there were about 8 per cent *lata*, 36 per cent *scintillans*, 11 per cent *cana*, 8 per cent *pallescens*, 4 per cent *liquida*, and 4 per cent *spathulata*. Of course, the figures are only approximate, since a large part of the plants did not reach the period of flowering, but it should be noted that the size of five of these groups lies between 4 and 11 per cent, whereas only one, that of *scintillans*, is seen to differ widely. The kind of pollen used for the crosses did not seem to have a notable influence on the numerical relations between these divisions.

Combining this result with our hypothesis concerning the distribution of the mutant characters among the six lateral chromosomes of the haploid nucleus (8), we find an exact correspondence in the main points, but it seemed desirable to give further proofs and to study these relations in more detail.

We shall first give a survey of the groups concerned, and discuss the individual deviations afterward. We call prototypes the forms of the old races, which served for comparison. It should be remarked that the mutants of our experiment cannot be expected to be exactly the same as these prototypes, since their male parent was always the *velutina*. In the prototypes the paternal half of the nucleus is *velutina* only in the sesquiple mutants *oblonga*, *auricula*, and *candicans*, but *laeta* in the dimorphic races (*lata*, *scintillans*, *cana*, *pallescens*, *liquida*, and *spathulata*). This causes some differences, especially in the leaves, which are broader for *lata* and narrower for *velutina*. In some instances this difference is striking, but in others it is very small, as in the case of *O. cana*.

The isogamic mutants *nanella* and *rubrinervis* have not been observed in our culture; no dwarfish or brittle specimens were seen. Some plants were like *Lamarckiana*, and others constituted a new type, which was almost like the parent species, but reached only half its height. We call it *O. Lamarckiana* mut. *pulla* (fig. 2). Two plants repeated the features of *O. Lamarckiana* mut. *hamata*, a race cultivated since 1918, but as yet not published. The vast majority of the mutants showed more or less clearly the types of our old dimorphic races. Table III gives the prototypes represented in the numbers of individuals.

From table III we see that the dimorphic races, with the exception of *lata* and *cana*, were represented in high and almost equal numbers (10-13.5 per cent), whereas the secondary types were present in only one or two specimens each. There are seven main groups, including *lata* but omitting *Lamarckiana*, and this points to the conception that each of them corresponds to one of the seven chromosomes of the haploid nucleus.

If we apply the law of probability not only to the numbers of the chromosomes, but also to their qualities, we may consider it very probable that the different chromosomes will be equally represented among the different groups of seedlings. Thus we should

TABLE III
GROUPS ACCORDING TO PROTOTYPES

Prototypes	Number	Percentage
<i>lata</i>	2	2.5
<i>scintillans</i>	10	12.5
<i>oblonga</i>	1	1.3
<i>auricula</i>	2	2.5
<i>cana</i>	18	22.5
<i>candicans</i>	1	1.3
<i>pallescens</i>	8	10.0
<i>liquida</i>	9	11.3
<i>spathulata</i>	11	13.5
<i>hamata</i>	2	2.5
<i>pulla</i>	10	12.5
<i>Lamarckiana</i>	6	7.5
Total.....	80

expect seven main groups of mutant characters to be represented with about equal frequency, each corresponding to the doubling of a distinct nuclear rod. The expected groups would then correspond to the observed ones. Why the type of *lata* should be rarer and that of *cana* more frequent remains obscure. In this respect, however, it should be remembered that among the progeny of *O. perennis* (*O. blandina* × *simplex*) *semigigas*, another group, that of the type of *scintillans*, is in the majority, a fact which obviously diminishes the importance of such deviations. From this we may infer that the different chromosomes are the bearers of distinct groups of mutant characters, or, in other words, that each chromosome is the bearer of the factors of one of the seven main types of table III. This conclusion is in harmony with the hypothesis of

the distribution of mutant characters among the chromosomes, and may be considered to offer a new proof for it. Moreover, in their main features the groups are the same as those described for *O. perennis*.

Table IV gives the number of chromosomes for the constituents of these different groups. It is to be remarked that one of the 81 plants (one with 17 chromosomes) died in early youth, before developing its character. All the others reached the fall, mostly in flowering condition, but partly as rosettes or young stems.

In almost every group at least one of the chromosomes is doubled, and the doubling of two or more such bodies, taken

TABLE IV
MUTANTS OF *O. semigigas velutina*

Prototypes	Number of chromosomes						
	14	15	16	17	18	19	20
<i>lata</i>		1		1			
<i>scintillans</i>		2	3	2	2	1	
<i>oblonga</i>		1					
<i>auricula</i>		1		1			
<i>cana</i>	10		3	3		1	1
<i>candicans</i>		1					
<i>pallescens</i>		1	3	1		2	1
<i>liquida</i>		7	2				
<i>spathulata</i>		4	4	2	1		
<i>hamata</i>		2					
<i>pulla</i> (fig. 2).....		5	3	2			
<i>Lamarckiana</i>	3	1					2

broadly, is equally distributed among them. From this we may conclude that the external features of the types as a rule are connected with the doubling of one chromosome in each case, but that the doubling of the others is of secondary importance. It produces, or is related to, small deviations from the prototype, but does not essentially change it. We may assume that in every case the factors of one chromosome are dominant over those of the others, but why in one case this chromosome should prevail, and others in other cases, we do not know. This condition, however, obviously runs parallel with the well known fact that the parent species produces in some instances a *lata*, in others a *scintillans* or a *cana*, and this without the slightest indication of the cause of such a choice.

In every group two main causes of individual differences are to be considered. One of them is connected with the doubling of more than one chromosome, and it is usually assumed that factors of the other doubled ones are influencing the visible features of the plants. Even if only one chromosome is doubled, however, our plants may show deviations from their prototype, and in such cases we must suppose that in the same rod different groups of factors have become mutated.

Pure types have occurred in our experiment only in specimens having one rod doubled, as will be seen in table V. In this table

TABLE V
PURE AND DEVIATING TYPES OF MUTANTS

PROTOTYPES	15 CHROMOSOMES		16-20 CHROMOSOMES	
	Pure	Deviating	Pure	Deviating
<i>lata</i>	1	1
<i>scintillans</i>	2	4
<i>cana</i>	8	2	5
<i>pallescens</i>	5
<i>liquida</i>	7	2
<i>spathulata</i>	4	(o)

only the flowering plants are mentioned, but it is to be remarked that for *spathulata* 6 specimens which did not produce flowers could be added to the last column. The specimens of *oblonga*, *auricula*, *candicans*, and *hamata* were sufficiently pure to their types, and of the *pulla*, for which no prototype is as yet known, all had the same form, with only one exception (with 16 chromosomes). It must be remarked, however, that numerical relations of this kind are very changeable among the progeny of *semigigas*, and that other crosses of it have given different results. It is, therefore, only on the main points that we wish to lay stress. Numerous types may lay hidden in the germs of the non-germinating seeds.

CHARACTERS OF MUTANTS

The different forms of mutants seen in our cultures will be described, beginning with the largest group, the *cana*-like plants. It is at the same time the most complete, and the incompleteness of the other groups may therefore be ascribed to their smallness.

PROTOTYPE *cana*.—Seven plants were exactly like the race of this name, which has been described by one of the writers (5), and of which the fourth generation flowered in 1923 in the experimental garden in 6 pure specimens. The likeness was already evident in the rosettes, and the 7 plants were planted in a row. By this means their uniformity was made very striking. The spikes are long and thin, with long internodes, narrow bracts, long thin flower buds, and thin cylindrical fruits. All organs are grayish. The flowers are almost erect, and the calyx tube is correspondingly bent. The petals spread only halfway. The tips of the calyx are bent sideways in the plane of symmetry of the buds, pointing outward in respect to the spike. All of the seven specimens reached the same height, which in mid August reached 1-1.25 m. They were richly branched. They only differed from the old race in somewhat narrower leaves and bracts, and slightly more compact flower buds, as was to be expected from the influence of their *velutina* parent. Among the deviating plants of this group two specimens with 15 chromosomes differed only in being weaker, reaching about one-half the height of the others, and in having thicker fruits, which were almost like those of *O. Lamarckiana*.

The three specimens of *cana* with 16 chromosomes hardly differed from the main type. Two of them had broader leaves, especially in the rosettes, the fruits being *cana*-like in one of them and *Lamarckiana*-like in the other. The third plant developed its grayish color relatively late in the spring. Their flowering spikes were as described, with the exception that the anthers of one specimen were almost barren. The two plants with 17 and 19 chromosomes were also evidently *cana*, one with broader and one with narrower leaves, and both with smaller and stouter flower buds. They were weak plants, which opened their first flowers only in the last days of August. Three specimens of the *cana* type stayed in the condition of rosettes.

There was one *candicans* among the plants. In early youth the race of this name is almost exactly like *cana*, and so this plant was considered to belong to this type until it developed its flowering spike, which proved it to be a *candicans*. Differences between this specimen and the race have not been noticed.

PROTOTYPE *lata*.—There were only two plants belonging to this type, one with 15 and one with 17 chromosomes. Both differed in many points from the pattern, as in having stout stems and shorter leaves. They did not reach the flowering period before the fall, and were mainly recognized by the dense grouping of their young leaves, so characteristic for *lata*.

PROTOTYPE *scintillans*.—In 1923 we had a large bed of *scintillans* × *blandina* in order to compare the mutants with this hybrid. The cross had been made in 1919, and the culture of 1923 was the second generation, which embraced about 20 specimens of the type of *scintillans*, with the same dark green and shining leaves, but considerably narrower than those of the race. This was especially evident during the period of the rosettes, and at this time four of the mutants were exactly like the hybrid prototype, which carried the characters of *O. Lamarckiana* mut. *velutina* (*O. blandina*) in the paternal halves of their nuclei. Of these four mutants, two had 15 chromosomes and the two others 17. The two first remained true to their hybrid pattern during their whole life, but of the two latter one distinguished itself at the flowering period by a broader foliage. The fourth one stayed in the condition of a rosette until the fall.

Among the remaining six specimens, three were narrow leaved and three broad leaved in comparison with the typical ones. The first had 16, 18, and 19 chromosomes, and the latter 16, 16, and 18. Sundry smaller differences were observed among them, as was to be expected, but the main type was in every case that of the group. One narrow leaved and one broad leaved plant have not succeeded in developing a stem in the first year (chromosomes 18 and 19).

One specimen of *oblonga* occurred. It was easily recognized when still very young, and remained throughout life true to its type. Of *auricula*, two specimens were observed (with 15 and 17 chromosomes). We had a culture of 16 flowering plants of the fourth generation of the race of that name for comparison. The rosettes are easily confused with those of *cana*, but the stout stems, large flower buds, and broad fruits cannot leave any doubt. Differences between the two mutants and the race have not been observed.

PROTOTYPE *pallescens*.—Only one plant of this group had 15 chromosomes. It was almost like the normal race, but had somewhat broader leaves, especially in its youth. Three specimens

had 16 chromosomes, one of them remained a rosette, whereas the two others flowered but produced hardly any pollen. The same condition prevailed for a plant with 17 and another with 19 chromosomes, and another plant with the latter number did not flower. Among the flowering plants of this group a curious deviation occurred more or less often, consisting in swollen stigma lobes which broadened the tops of the flower buds before opening. One plant with 20 chromosomes was free from this disease, and produced enough pollen for self-fertilization. It, however, had long, very narrow, and almost linear leaves.

PROTOTYPE *liquida*.—The plants of this group constituted a very uniform type, which could be compared with a culture of *O. liquida* × *Lamarckiana* mut. *velutina*, and was found to be almost identical with it, in the rosette stage as well as during the period of flowering. The root leaves are lanceolate, longer and narrower than those of the pure race of *liquida*, and pressed to the ground so as to form a dense rosette. The flower spikes were dense like those of the race, with numerous long and somewhat thin buds and stout fruits. In the culture of the race of *liquida* outside of the glass covered part of the garden the color was brownish, but in a specimen in that part and in the mutants the young organs were almost a pure green. Seven of the plants had only one chromosome doubled; in the two other plants two nuclear rods were in this condition. One of them did not produce a stem; it had long petioles and very pointed leaf blades; the other was almost equal to the typical specimens of the group. On the flowering plants the pollen was abundant, and artificial fertilization was always easy and successful.

PROTOTYPE *spatulata*.—Almost circular leaf blades on long and erect petioles are characteristic of this group during the stage of rosettes, and make it easily recognizable. This type of leaves is followed by the spatulate form as soon as the stem begins to develop. In the individuals with 15 chromosomes the root leaves were pale green; in the others they had a darker tinge and were usually more or less bent against the soil. As a rule the plants in this group were weak. Only those four which had 15 chromosomes flowered, but none of them has produced sufficient pollen for artificial self-fertilization. Among those with 16, 17, and 18 nuclear

rods, three could produce only low stems and four remained in the condition of rosettes.

In the outward features of their inflorescences, the four plants with the normal number of chromosomes were almost exactly like the specimens of the pure race of *O. spatulata*. We also had a

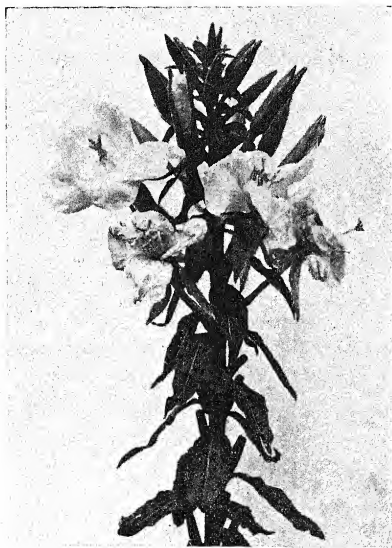


FIG. 2.—*Oenothera Lamarckiana* mut. *pulla*; photograph by M. STAKMAN.

biennial plant of *spatulata* \times *blandina*, which flowered richly, but did not show striking differences from the pure mutant.

The two plants of *hamata* were only recognized as such at the time of flowering, and could be compared with 15 typical specimens of the fifth generation of the pure, dimorphic race of the same name. No real differences were observed.

TYPE *pulla* (fig. 2).—Of the ten plants of this form, nine were almost exactly alike, with a very typical form of the radical leaves and the same stem and spike reaching the same height at the time of flowering, in the middle of August. One specimen with 16 chromosomes slightly deviated in its characters; moreover, it did not produce any good pollen, whereas all the others were rich in this respect. It was also weaker and of lower stature. The main form reached about one-half the height of the normal *Lamarckiana*, and had the same stature as this, with a more condensed spike and a less branched stem. Of all the mutants, the *pulla* differed the least from *O. Lamarckiana*. The flower buds reached the same size, although they were not as completely filled. The fruits were as broad and stout, and as rich in seeds as those of the parent species. In the spring the radical leaves of this form had been quite a puzzle, since the type was unknown at that time. These leaves resembled those of *O. gigas* in being broad, with a rounded tip and a sharp contraction of the bases, which separated the blade from the long and winged petiole. They were given a special name, but proved later to correspond to the type now called *pulla*.

On account of the small and hardly striking differences from the parent species, we assume that the factors of *pulla* are to be sought for in the same chromosome which decides the main characters of *O. Lamarckiana* itself, bearing the determiners for *laeta* in some of the gametes and those for *velutina* in others. We have called this chromosome the central one, but the outward features of *pulla* are accompanied by a doubling of one of the nuclear rods. Five specimens had 15 chromosomes, whereas three had 16, and two 17 such bodies. The mutations previously ascribed to the central chromosome, such as dwarfishness and brittleness, are not accompanied by an increase of the number of nuclear rods. If our conception is true, the *pulla* completes the series of the seven cases of chromatic doubling which must cooperate in the formation of *semigigas*. From this point of view it is interesting to note that the *pulla* occurred in about the same number of individuals (12.5 per cent) as most of the other main types of mutations.

Lamarckiana-LIKE PLANTS.—In the reduction division of *semigigas*, the extreme case is that the seven doubled chromosomes

go to one gamete and the seven single ones to the other. After fertilization with *O. (biennis* × *Lamarckiana*) *velutina* the first group would produce germs with 21, but the other group would give embryos with 14 nuclear rods. Those with 21 would constitute *semigigas* plants, but their chance of survival is small, and in our experiment such individuals have not occurred. Those with 14 chromosomes would give pure or almost pure *Lamarckiana*, provided their maternal gamete belonged to the *laeta* type. Such is probably the case at least in a sufficient number of germs, as we have previously seen in the crosses of the pollen of *semigigas*.

In the culture of the progeny of *semigigas* × *velutina*, three plants were found with 14 chromosomes and the external marks of *O. Lamarckiana*, corresponding to the expectation derived from this discussion. The rosettes were very stout and easily recognized, almost all the others being much weaker. In the flowering condition the stems were as high and as stout as in the species itself, but the flowers showed some influence of the characters of their grandparent *O. biennis*, as was to be expected. The flower buds were somewhat more cylindrical and the petals smaller than in the pure *Lamarckiana*. For the same reason the spikes were more compact and the bracts smaller, but the differences were very small and did not essentially lessen the resemblance to ordinary *Lamarckiana*.

Moreover, there were two plants with the same stature and external marks, which, however, had 15 and 20 chromosomes. In these cases the doubling of the chromosomes was not followed by the visible mutations which otherwise always seem to accompany it, proving once more that this combination is not a necessary one. Another plant with 20 chromosomes had very long, almost linear leaves, constituting a type of its own of very doubtful interpretation. The linear form of the leaves, however, although not at all rare among the progeny of *semigigas*, does almost always obscure the other marks.

Summary

1. The progeny of *Oenothera (lata* × *Lamarckiana*) *semigigas* × *velutina* constitutes a motley assembly of forms, many of which are already recognizable among the young seedlings. On the

contrary, the hybrids, produced by means of the pollen of *semigigas*, did not show any noticeable variability.

2. In the first named cross the mutants belong to different groups, corresponding to the older mutant races derived from *O. Lamarckiana*. There are seven main types, of which six are those of the dimorphic (heterogamous and splitting) mutants *lata*, *scintillans*, *cana*, *pallescens*, *liquida*, and *spathulata*, while one constitutes a new type (*O. mut. pulla*). With the exception of *lata* and *cana*, these groups occurred with almost the same frequency.

3. Besides these main forms, single plants of secondary types were observed. They were one *oblonga*, two *auricula*, one *candicans*, and two *hamata*.

4. In almost one-half of the mutants (35 individuals out of 81) the number of chromosomes was 15, or the same as in the older heterogamic mutant races. As a rule, these plants showed all the marks of those races, although, on account of their *velutina* gametes, they could not be expected to be identical with them.

5. In the other half of the mutants the chromosome numbers were 16-20, and the external features were correspondingly mixed, but almost always one of the main types could be recognized as the prevailing one.

6. The 15 chromosome offspring of *semigigas* belong to different types. Among these some are frequent and others rare. The frequent types are seven in number, and in each of them a different chromosome must be doubled. Of the rarer ones each must also have one chromosome doubled, but in these cases other factors of the same rod must have been activated.

7. Among all the mutants, the new form *O. pulla* shows the least deviation from the original form *O. Lamarckiana*. It is therefore assumed to have its factors located in the central chromosome. The remaining six types are those of the old dimorphic races, each of them designating a lateral chromosome, as had already been deduced by us from other considerations. The rarer types are such as have previously been pointed out as secondary ones. It is assumed that *oblonga* and *auricula* have their factors in the *scintillans* chromosome, whereas *candicans* is derived from *cana* and *hamata* probably from *spathulata*.

8. In order to get sufficiently sharply distinguished types among the progeny of *semigigas*, some conditions must be fulfilled. In the first place, *velutina* pollen should be used for the fertilization, since its qualities, as a rule, are recessive to those of the mutants. Then the culture must be made under favorable conditions, as in the glass covered part of the garden. Also, the young seedlings should be planted singly in small pots, and selected and arranged shortly before being transplanted on the beds. If this is done the mutants belonging to the same type will flower next to one another, thereby giving a striking survey of the uniformity of their main features.

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NEW AMERICAN MEIBOMIAS

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(WITH PLATE IV)

The late JOSEPH H. PAINTER of the United States National Herbarium undertook a monograph of the American species of *Meibomia* (*Desmodium*) a number of years ago, but never carried it far. At intervals during several years the writer has been occupied in the attempt to carry out a revision of this genus, but the pressure of other work has compelled the abandonment of the plan. A number of species described by the older investigators, and not recognized in recent years, have been found in the material examined, and it has been possible to reduce a number of names to certain or probable synonymy. So many of the earlier species are inadequately described, however, that an examination of several European herbaria is necessary to settle their identity, and a thorough revision of the genus is not practicable at the present time. The present paper, therefore, is limited to the description of a dozen new species from Mexico and Central and South America, and the discussion of a few other forms.

In addition to the large amount of material in the National Herbarium, I have examined, through the kindness of Dr. N. L. BRITTON and Dr. B. L. ROBINSON, several sheets from the New York Botanical Garden, and a large number from the Gray Herbarium, which have been of great assistance in the prosecution of the work. In the following descriptions, the measurements of the calyx lips are taken from the base of the calyx; those of the loment include the stipe when present. Unless otherwise cited, all specimens listed are in the United States National Herbarium.

Meibomia pycnantha, sp. nov.—Shrub, 1.6–5 m. high, branching; stem appressed-pilose, glabrescent, leafy; stipules medium-sized, deciduous; leaflets 3, elliptic, large, accumbent-pilose and at first sericeous beneath; racemes axillary and terminal, short, very densely flowered, spikelike, comose; bracts suborbicular-ovate, rather abruptly short-acuminate; flowers medium, short-pedicelled;

calyx silky-pilose; loment of *Heteroloma* type, stipitate, straight or curved, flat, pilosulous, the joints 2-4, obliquely oval or ovate-oval, about 7 mm. long. Fig. 2.

Stem rather stout, purplish brown, at first densely appressed- or ascending-pilose; stipules lanceolate, 6 mm. long, acuminate brown, appressed-pubescent; stipels narrowly subulate, quickly deciduous, 3 mm. long; petiole (1.2-4 cm. long) and rachis (8-22 mm.) appressed- or ascending-pilose; petiolules 2-4 mm. long; leaflets elliptic or narrowly ovate-elliptic, obtuse, mucronulate, rounded at base, papery, above dull green, evenly accumbent-pilose (hairs with slightly enlarged bases), beneath paler, at first densely silky-pilose, at maturity evenly accumbent-pilose, densely so along costa, prominulous-reticulate beneath in age (lateral veins about 8 pairs), terminal one 3.5-9.5 cm. long, 1.5-3 cm. wide, lateral about one-quarter smaller; racemes at first conical, densely bracteate, in bud and anthesis 1.5-2.5 cm. long, at maturity 5.5 cm. long or less, short peduncle (about 5 mm. long) and axis densely silky-pilose; bracts deciduous after anthesis, firm, purplish-tinged, striatulate, pilose-ciliate and appressed-pilose toward base with white hairs, body suborbicular-ovate, 3-4 mm. long, acumination 1-2.5 mm. long; pedicels paired, pilose, 3-4 mm. long, decurved in fruit; calyx 4 mm. long, lobes all subequal, upper lip entire, obtuse, lobes of lower lip triangular, acute; corolla with some purple, pale when dried, banner 1 cm. long; stipe of loment 3-4.5 mm. long, exceeding the withered calyx; loment 1.8-3.5 cm. long, shallowly or rather deeply lobed on upper margin, much more deeply so on lower, isthmi short or sometimes 1 mm. long; joints whitish, reticulate, evenly but sparsely curved-pilosulous on surface, more densely so on margin and there sometimes finely uncinat-hispidulous, 6-9 mm. long, 4-6 mm. wide.

MORELOS.—Mountain side above Cuernavaca, altitude 2440 m., February 2, 1899, Pringle 8046 (type no. 1,166,602, U.S. Nat. Herb.).

STATE OF MEXICO.—Dry hills, Salto de Agua, November, 1905, Purpus 1744.

This fine species is a member of the *Meibomia amplifolia* group, allied to *M. densiflora* (Hemsl.) Kuntze and *M. consattii* (Greenm.) Standl. It is easily distinguished from *M. consattii* by its densely pilose ovary (in *M. consattii* merely pilosulous) and the much shorter acuminations of the bracts, these being 3-6 mm. long in *M. consattii*. No material agreeing with HEMSLEY'S descrip-

tion of *Desmodium densiflorum* has been examined, but his description applies so well in nearly all details to *M. consaztii* as to lead to the suspicion that the two are identical. The chief discrepancies are in the flowers, which are described as sessile by HEMSLEY, and the ovary, which is described as glabrous. The type collection of *M. pycnantha* was distributed without name, and PURPUS' plant as "*Desmodium? densiflorum*."

Meibomia sylvestris, sp. nov.—Herbaceous or suffrutescent, branching; stem finely uncinat-puberulous and often sparsely hirsute, glabrate or glabrescent; stipules deciduous; leaves slender-petioled; leaflets 3, ovate, antrorse-pilose on both sides, sometimes glabrate above; racemes usually paniced, elongate, loose; bracts small, deciduous; pedicels 1-2 cm. long; flowers medium; loment of *Heteroloma* type, subsessile or stipitate, the 3-6 joints usually half-rhombic, densely uncinat-hirsutulous, 7-10 mm. long. Fig. 1.

Stem rather slender, terete, up to 2 m. high, usually glabrate; stipules subulate-attenuate, about 6 mm. long; stipels bristleform, 2-4 mm. long; petiole (2-4.5 cm. long) and rachis (6-17 mm.) hirsutulous or glabrate; leaflets ovate, varying to elliptic-ovate or broadly ovate, obtuse, mucronulate, rounded at base, thin, sometimes becoming pergamentaceous in age, above deep green, evenly but not densely antrorse-pilose (hairs with small tuberculate bases), sometimes glabrate, beneath similarly pubescent, prominulous-reticulate beneath in age, terminal one 3.2-7.5 cm. long, 2-4 cm. wide, lateral usually about one-third smaller; racemes peduncled, up to 25 cm. long, axis densely uncinat-hispidulous and sparsely hirsute; pedicels usually paired, uncinat-puberulous, spreading or ascending in fruit; bracts lance-subulate, 4.5 mm. long, striate, flavescent-hirsute; calyx flavescent-hirsute and minutely puberulous, upper lip bidentate, 4.8 mm. long, lower 5.8 mm. long; corolla "violet," banner 11 mm. long; stipe of loment usually 2-5 mm. long and equaling or exceeding the withered calyx, sometimes obsolete; loment straight, shallowly lobed on upper margin, deeply so on lower, 2.8-6.2 cm. long; joints half-rhombic or sometimes nearly oval, golden brown in age, 6-10 (rarely 14) mm. long, 5-6 mm. wide.

COLOMBIA.—In forest, Cordillera Oriental, east of Neiva, Dept. Huila, altitude 1800-2300 m., August 1-8, 1917, *Rusby & Pennell* 577 (type in herbarium of N.Y. Bot. Gard.; fragment in U.S. Nat. Herb.); forest slope,

Tequendama, Dept. Cundinamarca, altitude 2200-2300 m., September 15, 1917, *Pennell* 1980; in forest above Fusagasuga, Dept. Cundinamarca, altitude 1800-2300 m., November 28-29, 1917, *Pennell* 2690; edge of forest above Saliento, Dept. Caldas, altitude 2100-2500 m., July 25-31, 1922, *Pennell* 8885.

Related to *Meibomia orizabana* (Hemsl.) Kuntze, which has a nearly or quite simple, usually strongly angled and essentially glabrous stem, acuminate and nearly or quite glabrous leaflets, and a non-hirsute calyx. *Meibomia orizabana*, originally described from the region of Orizaba, Veracruz, occurs also in Oaxaca (*Consatti* 3819), Chiapas (*E. W. Nelson* 3257), Costa Rica (*Pittier* 16614, *Tondus* 12628), and Colombia (Popayan, *Lehmann* [no. 5114 in N.Y. Bot. Gard.; no. B. T. 376, in Gray Herb.]; Dept. El Cauca, *Pennell & Killip* 8049; Dept. Caldas, *Pennell* 10661, 10729; Dept. Antioquia, *Pennell* 10791). The detailed description of *Hedysarum caripense* H.B.K. (= *Meibomia caripensis* [H.B.K.] Kuntze), from Caripe, Cumaná, agrees so well with *M. orizabana* as to render it almost certain that the two species are identical.

One of *PENNELL*'s specimens of *M. orizabana* (no. 10729) shows a regularly pinnate 5-foliolate leaf, in addition to normal 3-foliolate leaves, and another leaf of the same specimen is 4-foliolate, the extra leaflet in this case being much smaller than the other three, and borne at the apex of the rachis beside the terminal leaflet. I have seen no other specimens of *Meibomia* with more than 3 leaflets, but *BENTHAM* states in the *Genera Plantarum* that the leaves are very rarely 5-foliolate, and *HARMS* (Repert. Sp. Nov. Fedde 10:132. 1911) mentions several species with 5-foliolate leaves from New Guinea and New Caledonia.

Meibomia tephrophylla, sp. nov.—Shrub; stem uncinately hispidulous and hirsute-pilose; stipules lance-subulate, 4 mm. long, subsistent; leaflets 3, usually linear-oblong, obtuse to broadly rounded, sparsely antrorse-pilose above, more densely so beneath, grayish green; racemes paniced, about 15 cm. long; bracts small; pedicels about 5 mm. long; loment of *Heteroloma* type, stipitate, straight, cinereous-pilosulous with straight hairs, the 2-3 joints obliquely obovate or semiorbicular, often obscurely indented above, about 10 mm. long, 6 mm. wide. Fig. 6.

Stem slender, terete, apparently simple below inflorescence, densely uncinately hispidulous with spreading hairs and less densely hirsute-pilose with straight, spreading, ascending, or sometimes subappressed hairs; stipules dry, ciliate, persistent or deciduous; stipels very narrow, persistent, 1-2 mm. long; petiole (6-10 mm. long) and rachis (4-8 mm.) deeply grooved above, pubescent like the stem; petiolules 1-2 mm. long; leaflets linear-oblong, elliptic-oblong, or lower sometimes lance-ovate or oval-ovate, apiculate,

rounded or subcordate at base, firm, lateral veins (4-7 pairs) scarcely prominulous above, prominent beneath, secondaries loosely prominulous-reticulate beneath, terminal leaflet 3.5-5.8 cm. long, 8-14 mm. wide, lateral about half as large; racemes several, subsessile, rather loosely flowered, axis and pedicels densely uncinat-hispidulous; bracts deciduous, not well seen; pedicels in clusters of 1-4, 3.5-5 mm. long, spreading in fruit; calyx hirsute-pilose and hispidulous with straight hairs, upper lip bidentate, 3 mm. long, lower lip 3.5 mm. long, its lobes acuminate, middle one longest; corolla purple, 7.5 mm. long; stipe of loment 4-5 mm. long, greatly surpassing the withered calyx; loment 2.2-3 cm. long, rather densely cinereous-pilosulous, especially on margin, shallowly lobed above, deeply so below, joints 9-12 mm. long, 5-6.5 mm. wide.

JALISCO.—Barranca de Guadalajara, May 1, 1894, Pringle 5880 (type no. 316751, U.S. Nat. Herb.).

The material examined consists of a portion of leafy stem and two defoliate portions of stem bearing flowers and fruit. The species is evidently one of the *M. cajanifolia* group, nearest *M. cinerea* (H.B.K.) Standl., which has much broader and more pubescent leaflets and much smaller joints.

Meibomia ciliaris (Muhl.) Blake.—*Hedysarum ciliare* Muhl.; Willd. Sp. Pl. 3:1196. 1803; *Desmodium ciliare* DC. Prodr. 2:329. 1825.

In our manuals this species is given a range from Massachusetts to Florida, west to Ontario, Michigan, Missouri, and Texas. It occurs also in Cuba¹ (*Wright* 2318, from Pinales, Caimito,² September 13; Gray Herb.) and Haiti.³ Hitherto it has not been recorded from Mexico, and its occurrence so far south as Oaxaca, without intermediate stations, is very surprising. There is a sheet in the National Herbarium (no. 1,166,601), bearing three specimens in flower and fruit, collected by E. W. NELSON (no. 2694) near Santo Domingo, Oaxaca, altitude 485 m., June 18, 1895, which the most careful comparison has failed to distinguish in any character from *M. ciliaris*. It is true that all the stems terminate in simple racemes, while every specimen of *M. ciliaris* examined from other localities is more or less branched; but this is not likely to be more than an individual variation, and is covered by current descriptions of *M. ciliaris*. The possibility of error in the association of label and specimens

¹ GRISEBACH, Cat. Pl. Cub. 73. 1866.

² From the dates and other information given in UNDERWOOD's paper on WRIGHT's explorations in Cuba, Bull. Torr. Bot. Club 32:291-300. 1905, it is evident that this locality is in either Matanzas or Habana.

³ URBAN, Symb. Antill. 8:293. 1920. Specimens collected near Furcy by LEONARD (no. 4750) are in the National Herbarium.

is obviated by the fact that to one of the stems is still tied the original collecting tag, bearing the number corresponding to the label.

For thirty years American investigators, following VAIL,⁴ have united *Hedysarum obtusum* Muhl. and *H. ciliare* Muhl. under the name *Meibomia obtusa* or *Desmodium obtusum*, the two names of MUHLBERG being considered synonymous, and *H. obtusum* having page priority. TORREY and GRAY⁵ were in some doubt as to the proper reference for the type of *H. obtusum* in the WILLDENOW herbarium, but considered that it was probably a smoother variety of *D. ciliare*, rather than a form of *D. marilandicum*. URBAN⁶, however, has recently used the name *Desmodium ciliare*, remarking that the types of *H. ciliare* and *H. obtusum* in the WILLDENOW herbarium appear to be very different plants. Under these circumstances it seems best to readopt the name *ciliare*, the description of which is unequivocal. *H. obtusum* will probably prove to be a form of *M. marilandica*, as the description suggests, and as the earlier investigators interpreted it.

Meibomia cana (Gmel.) Blake.—*Hedysarum racemosum* Aubl. Pl. Guian. 2:774. 1775; *H. supinum* Swartz, Prodr. Veg. Ind. Occ. 106. 1788 (not *Hedysarum supinum* Chaix; Villars, Prosp. 41. 1779-80); *H. incanum* Swartz, Prodr. Veg. Ind. Occ. 107. 1788 (not *H. incanum* Thunb. Fl. Jap. 289. 1784); *H. canum* Gmel. Syst. Veg. 2^a:1124. 1791; *H. racemiferum* Gmel. Syst. Veg. 2^a:1125. 1791; ?*Desmodium racemiferum* DC. Prodr. 2:331. 1825; *D. incanum* (Swartz) DC. Prodr. 2:332. 1825; *D. supinum* (Swartz) DC. Prodr. 2:332. 1825; *Meibomia adscendens* β. *incana* (Swartz) Kuntze, Rev. Gen. Pl. 1:195. 1891; *M. racemifera* (DC.) Kuntze, Rev. Gen. Pl. 1:198. 1891; *M. supina* (Swartz) Britton, Ann. N.Y. Acad. Sci. 7:83. 1892; *Desmodium canum* (Gmel.) Schinz & Thellung, Mém. Soc. Neuchât. Sci. Nat. 5:371. 1914.

The principal synonyms of this much-named species are given in this list. *Hedysarum racemosum* Aubl., *H. canum* Gmel., and *H. racemiferum* Gmel. are all based primarily on a figure in BURMANN's edition⁷ of PLUMIER. This figure represents the plant usually known as *Desmodium incanum*, *D. supinum* or *Meibomia supina*, the acute-elliptic or oval-elliptic leaflets and the persistent bracts of the raceme being definitely those of *M. supina* and not of *M. adscendens* (Swartz) Kuntze. The earliest name, *Hedysarum racemosum* Aubl., is not available, being preoccupied under *Meibomia* and *Desmodium* by *M. racemosa* (Thunb.) Kuntze and *D. racemosum* (Thunb.) DC., both based on *H. racemosum* Thunb. (1784). *Hedysarum supinum* Swartz is preoccupied by *Hedysarum*

⁴ Bull. Torr. Bot. Club 19:115. 1892.

⁵ Fl. N. Amer. 1:362. 1840.

⁶ Symb. Antill. 8:292. 1920.

⁷ PLUMIER, Pl. Amer. ed. BURMANN 140. pl. 149. fig. 1. 1757.

supinum Chaix ex Villars,⁸ and *H. incanum* Swartz by *H. incanum* Thunb. The first available name, *Hedysarum canum* Gmel., must be taken up for the species.

The description of *Hedysarum guianense* Aubl. somewhat suggests this species, but the type in the British Museum is *M. barbata* (L.) Kuntze, as I have been informed by Mr. E. G. BAKER.

Meibomia acuminata (Michx.) Blake.—*Hedysarum acuminatum* Michx. Fl. Bor. Amer. 2:72. 1803; *H. glutinosum* Muhl.; Willd. Sp. Pl. 3:1198. 1803; *Desmodium acuminatum* DC. Prodr. 2:329. 1825; *Desmodium grandiflorum* and *Meibomia grandiflora* Amer. auth. (not *Hedysarum grandiflorum* Walt. 1788).

This very distinct species was almost universally known as *Desmodium acuminatum* until 1891, when OTTO KUNTZE adopted for it, without discussion, the name *Meibomia grandiflora* (Walt.) Kuntze,⁹ based on *Hedysarum grandiflorum* Walt.¹⁰ WALTER's plant, which is not mentioned by MICHAUX, PURSH, ELLIOT, TORREY and GRAY, or any other of the earlier writers on North American plants, and which was listed by DECANDOLLE among the "species trifoliata non satis notae" as *Desmodium grandiflorum*, was apparently first noticed again by SERENO WATSON, who cited it (Bibl. Ind. N. Amer. Bot. 215. 1878) as a synonym of *Desmodium acuminatum*. It was probably on the basis of this reference that KUNTZE was led to adopt the name in this sense.

Aside from the fact that the name *grandiflorum* is preoccupied in *Hedysarum* by *H. grandiflorum* Pall. (Reise 2:743. 1773), and so is not available under the American Rules, it is clear from WALTER's description, quoted below that he could not have referred to the plant under consideration. The species now passing as *Desmodium grandiflorum* or *Meibomia grandiflora* has the leaves crowded at the summit of a stem about a foot long, which bears at its apex a single, naked, long-peduncled raceme or panicle; the leaflets are not noticeably veiny, the lomenta are 2- or 3-jointed, and the flowers are not notably large. WALTER's description, however, calls for a plant with veiny leaves, axillary racemes, many-jointed lomenta, and large flowers. WALTER's fragmentary type is still preserved in his herbarium at the British Museum, where it has been examined for me and compared with authentic material by Mr. E. G. BAKER. Mr. BAKER reports that the type has leaflets glabrous beneath, conspicuous and persistent stipels, ovate, acuminate, strongly striate bracts, and uncinately

⁸ Mr. T. F. CHIPP, Assistant Director of the Royal Botanical Gardens at Kew, has kindly forwarded a copy of VILLARS' description, taken from the copy of his work in the library of the Linnean Society. VILLARS' work is very rare and apparently is not in any American library.

⁹ Rev. Gen. Pl. 1:196. 1891.

¹⁰ WALTER, Fl. Carol. 185. 1788: "*grandiflorum* 6. fol. ternatis ovalibus venosis utrique laevibus; racemis axillaribus erectis, leguminibus pendulis multiarticulatis, floribus majoribus."

hirsutulous inflorescence. The specimen shows no stipules, and is too small to show whether the leaves are scattered along the stem.

In all the positive characters mentioned, WALTER's type disagrees with the plant now passing as *Meibomia grandiflora* or *Desmodium grandiflorum*, but does agree with *M. bracteosa*. WALTER's description, moreover, applies well to this species, which has the largest flowers of any eastern species except *M. canadensis*. Several specimens of these two species (*M. bracteosa* and *M. "grandiflora"*) were sent to Mr. BAKER, who states that a specimen of *M. bracteosa* (Standley 9809) agrees best with WALTER's type.

From the information supplied by Mr. BAKER, as well as from WALTER's own description, it is clear that the names *Meibomia grandiflora* (Walt.) Kuntze and *Desmodium grandiflorum* (Walt.) DC. must now be transferred to the plant at present called *Meibomia bracteosa* (Michx.) Kuntze or *Desmodium bracteosum* (Michx.) DC, and that MICHAUX's name *acuminatum* must be adopted once more for the plant to which WALTER's name has been misapplied for a generation.

***Meibomia rhynchodesma*, sp. nov.**—Suffrutescent(?), scandent; stem uncinat-hirsutulous; leaves slender-petioled; leaflets 3, ovate, large, glabrate above, rather densely whitish-pilosulous beneath; panicles large, loose; flowers small; loment of *Nephro-meria* type, stipitate, rufid-pilosulous on margin, somewhat puberulent on surface; joints 1-2, suborbicular, about 1.8 cm. long, strongly inflexed and beaked at apex, upper margin notched, its length half to two-thirds that of the middle of the body. Fig. 3.

Stem slender, subterete, flexuous, flavescent-hirsutulous with mostly ascending uncinat hairs and minutely puberulent, glabrescent; stipules and stipels deciduous, not seen; petiole (2.5-4.5 cm. long) and rachis (about 1.8 cm.) sparsely uncinat-hispidulous; petiolules 2-3 mm. long; leaflets ovate or broadly ovate, acute or obtusish, rounded at base, papery, above dull green, beneath paler and prominulous-reticulate (lateral veins 4-5 pairs), terminal one about 8 cm. long, 5-6.2 cm. wide, lateral 5.5-7 cm. long, 3-4 cm. wide; panicles axillary, uncinat-hirsutulous, branches wide-spreading; bracts deciduous, not seen; pedicels solitary or paired, uncinat-puberulous, 3-7 mm. long; calyx short-ciliate, sparsely hirsute on lowest lobe, upper lip bidentate, 3 mm. long, lower lip 4 mm. long, lobes ovate or oblong-ovate, apiculate, middle one longest; corollas about 6 mm. long, not well seen; stipe of loment 3-5 mm. long, much exceeding the withered calyx; loment 1.3-2.5 cm. long on upper margin, shallowly lobed on upper margin, very

deeply on lower; joints (submature) 1-1.2 cm. long on upper margin, 1.3-1.9 cm. long across middle of body, 1.6-1.9 cm. wide, densely rufid-pilosulous on margin, on body puberulous and sparsely pilose, reticulate, upper margin with a notch 1-2 mm. deep, seed borne somewhat above the center.

COSTA RICA.—In thickets, Tuis, altitude 650 m., November 1897, *Tondus* 11448 (type no. 694364, U.S. Nat. Herb.).

A member of the *Nephromeria* group, related to *M. macrodesma* Blake and *M. purpusii* (T. S. Brandeg.) Blake. Both *M. macrodesma* and *M. rhynchodesma* differ from *M. purpusii* in having the upper margin of the joints of the loment only half to two-thirds as long as the central portion of the body. From *M. macrodesma*, *M. rhynchodesma* is distinguished by the dull white (not rufid) pubescence of the leaves and the smaller, merely puberulous loment joints, which at apex are prominently inflexed and beaked.

Meibomia crotalum, sp. nov.—“Climbing herb”; stem uncinat-hirsute; stipules mostly deciduous; leaflets 3, ovate, obtuse, large, persistently puberulous above, densely and softly pilose beneath; racemes paniced; flowers rather small; loment of *Nephromeria* type, short-stiped or subsessile; joints 1-2, reniform-suborbicular, ciliolate, about 2-2.5 cm. long, slightly beaked, upper margin notched, its length two-thirds to three-fourths that of the middle of the body. Fig. 4.

Stem slender, branched, densely uncinat-hirsute with rufescent hairs and sometimes densely and finely pilosulous, at length glabrate; stipules triangular-ovate, striate, hirsute-ciliate, about 7 mm. long, erect, at length brown and deciduous; stipels about 3 mm. long, often deciduous; petiole (2.5-4 cm. long) and rachis (8-16 mm.) densely hirsute-pilose with straight or hooked reddish brown hairs; petiolules 2-3 mm. long; leaflets ovate or broadly ovate, obtuse or rounded, mucronulate, broadly rounded to subcordate at base, papery, above dull green, beneath densely antrorse-pilose with grayish hairs, along the chief veins rufescent-pilose, feather-veined (chief veins 4-7 pairs) and in age definitely prominulous-reticulate beneath, terminal one 4.5-9 cm. long, 2.5-6 cm. wide, lateral about one-quarter smaller; racemes loose, axillary and terminal, up to about 20 cm. long, pubescent like the stem; bracts ovate, 4-5 mm. long, acuminate, striate, hirsute, deciduous; pedicels paired, spreading in fruit, uncinat-hirsutulous, 6-9 mm. long; calyx thin, hirsute and hirsutulous, upper lip 3.5 mm. long, acutely bidentate, lower

4 mm. long, the 3 lobes acuminate, middle one much the longest; corolla "violet-blue" or "purple-violet," banner 6 mm. long; stipe of loment 1-3 mm. long, about equaling the withered calyx, usually stout; pod straight, 1.5-3.8 cm. long on upper margin, slightly lobed on upper margin, very deeply so on lower, isthmus marginal, 1.5-3 mm. wide, joints broadly overlapping; joints greenish-white, reticulate, rufid-ciliate especially on lower margin, glabrous on sides or obscurely puberulous in region of seed, usually very slightly beaked at apex, deeply notched on upper margin, 1.3-2 cm. long on upper margin, 2-2.8 cm. long across middle of body, 1.9-2.5 cm. wide.

COLOMBIA.—In thickets, Cuchilla, east of Zarzal, Dept. El Valle, Cauca Valley, altitude 1100-1400 m., July 22, 1922, F. W. Pennell, E. P. Killip, & T. E. Hazen 8531 (type no. 1,143,492, U.S. Nat. Herb.); along stream, La Cumbre, Dept. El Valle, Cordillera Occidental, altitude 1500-1600 m., May 21-25, 1922, Pennell & Killip 5976; in forest, Tabeja, west of Armenia, Dept. Caldas, Cauca Valley, altitude 1100-1300 m., July 23, 1922, Pennell, Killip, & Hazen 8631; open land near Rio San Joaquin, La Gallera, Micay Valley, Dept. El Cauca, Cordillera Occidental, altitude 1400-1500 m., June 29-30, 1922, Killip 7854.

Allied to *M. metallica* Rose & Standl., *M. albonitens* (Lem.) Rose & Standl., *M. purpurea* (T. S. Brandeg.) Blake, *M. macrodesma* Blake, and *M. rhynchodesma* Blake; distinguished by its short-stiped loment, which is nearly or quite glabrous except on margin, and its blunt leaflets, which are densely puberulous above and densely soft-pubescent beneath. *Meibomia crotalum* is the first species of its immediate group to be found in South America.

Meibomia infracta (DC.) Blake.—*Desmodium infractum* DC. Prodr. 2:330. 1825; Moc. & Sessé, Calques des Dessins pl. 270; *Hedysarum biarticulatum* Moc. & Sessé; DC. Prodr. 2:330. 1825, as synonym (not *H. biarticulatum* L. 1753); *Desmodium barclayi* Benth. Bot. Voy. Sulph. 83. 1844; *Meibomia barclayi* Rose & Standl. Contr. U.S. Nat. Herb. 16:216. pl. 51, fig. e. 1913.

An earlier name for *Desmodium barclayi* Benth. is found in *D. infractum* DC., based on plate 270 of the Calques des Dessins of MOCINO & SESSÉ, drawn from a plant from "Mexici montibus." BENTHAM, when describing *D. barclayi*, recognized its relationship to *D. infractum*, but inferred a difference in the shape of the loment joints. Examination of the reproduction of MOCINO & SESSÉ's plate, in connection with DECANDOLLE's description, leaves no doubt of the identity of the plants concerned. HEMSLEY (Biol. Centr. Amer. Bot. 1:275. 1880) recorded *D. barclayi* from Guatemala, Nicaragua, Panama, and Venezuela. In their revision of the *Nephromeria* group, ROSE and STANDLEY

were able to cite material only from Costa Rica. The following specimens, all in the National Herbarium unless otherwise noted, have been examined by the writer:

VERACRUZ.—Puente Nacional, *Purpus* 6089 (fl.).

MICHOACÁN or GUERRERO.—La Correa, *Langlassé* 418 (fl.); La Botella, *Langlassé* 658 (fr.; Gray Herb.; ident. by MICHELI as *D. scutatum*).

OAXACA.—Guatulco, *Liebmann* (fr.); Cafetal Concordia, *Reko* 3362 (fl., fr.); District of Tuxtepec, *Consatti* 3757 (fr.).

HONDURAS.—Isla Tigre, *Standley* 20735 (fr.).

SALVADOR.—Vicinity of Ahuachapán, *Standley* 19815 (fr.).

COSTA RICA.—Nicoya, *Tondus* 13577 (fr.).

COLOMBIA.—Vicinity of Cartagena, 1919, *Bro. Heriberto* 245 (fl., fr.).

VENEZUELA.—Colonia Tovar, *Fendler* 1790 (fr.; Gray Herb.).

ECUADOR.—Guayaquil, 1923, *H. E. Anthony* 76 (fr.).

Meibomia psilacra, sp. nov.—Herbaceous, branched; stem densely uncinat-hirsutulous, inflorescence short-pilose with glandular-based hairs; stipules mostly persistent, lance-ovate, about 6 mm. long; leaflets 3, ovate, hirsute and hirsutulous especially toward margin and uncinat-hispidulous; racemes axillary and terminal, loose, elongate; bracts small, deciduous; pedicels spreading or ascending, 1–1.5 cm. long; flowers small; loment of *Chalarium* type, short-stipitate or subsessile, the 1–5 joints oval, about 9 mm. long, terminal one hispidulous on margin, otherwise essentially glabrous, others uncinat-hispidulous on margin and surface. Fig. 7.

Perennial, 30–60 cm. high; stem slender, subterete, green or purplish, densely uncinat-hirsutulous with ascending to deflexed hairs and finely puberulent; stipules stiff, acuminate, striate, purplish, erect or reflexed in age; stipels bristleform, about 1.5 mm. long; petiole (7–17 mm. long) and rachis (4–8 mm.) pubescent like the stem; petiolules 1–2 mm. long; leaflets obtuse or acute, mucronulate, rounded at base, pale green, firm-papery, above sparsely hirsute and somewhat uncinat-hirsutulous, toward margin densely hirsutulous with straight hairs, beneath similarly pubescent and prominulous-reticulate (lateral veins about 5 pairs), terminal one 1.8–3.8 cm. long, 1–2.2 cm. wide, lateral usually about half as large; racemes peduncled, paniced above, 10–30 cm. long, axis and pedicels short-pilose with spreading hairs with glandular yellowish bases, and finely puberulous; bracts subulate-ovate, 3 mm. long, acuminate, hirsute and puberulous, greenish; pedicels solitary or paired; calyx hirsute and puberulous, upper lip bidentate, 3.2 mm. long,

lower 4.5 mm. long, lateral lobes oblong, obtuse, middle one longer, triangular-ovate, acute; corolla purplish, about 8 mm. long; stipe of loment 1-2 mm. long, equaling or surpassing the withered calyx; loment equally lobed on both margins or slightly more deeply lobed beneath, straight or curved, flat or often somewhat twisted, 1.2-3.5 cm. long; joints usually 3 or 4, rarely 1 or 5, thin-walled, prominulous-reticulate, sometimes indented on upper margin, often (especially proximal joints) with somewhat inflexed margins, 8-10.5 mm. long, 6-7 mm. wide, all but terminal one rather sparsely uncinat-hispidulous on surface, densely so on margin, terminal one hispidulous with mostly straight hairs on margin, its body shining and nearly or quite glabrous.

SINALOA.—Sandy soil along river, near Fuerte, March 25, 1910, *J. N. Rose*, *P. C. Standley*, & *P. G. Russell* 13475 (type no. 636300, U.S. Nat. Herb.); dry hillside west of Álamos, March 16, 1910, *Rose*, *Standley*, & *Russell* 12960.

Allied to *Meibomia psilocarpa* (A. Gray) Kuntze, in which the loment is glabrous throughout except for some uncinat hairs on the margin.

Meibomia bella, sp. nov.—Shrubby, tall; stem strigose and finely pulverulent, inflorescence uncinat-hispidulous; stipules subulate-attenuate, reflexed, deciduous; leaves slender-petioled; leaflets 3, elliptic-ovate or ovate, prominulous-reticulate, firm, sparsely pubescent or glabrous above, sparsely strigose or antrorse-pilose on veins beneath; racemes numerous, paniced, comose at apex; bracts ovate, acuminate, 6-8 mm. long; pedicels about 1 cm. long; flowers large; loment of *Chalarium* type, stipitate, straight or usually somewhat twisted, sparsely crisped-puberulous, the 3-8 joints obliquely oval, about 3.5 mm. long. Fig. 10.

Branching, 2-3 m. high; stem slender, terete, usually purplish, densely pulverulent and less densely strigose, without hooked hairs below the inflorescence; stipules brownish, 4-7 mm. long; stipels bristleform, 2-3.5 mm. long, sometimes deciduous; petiole (2-5.5 cm. long) and rachis (5-13 mm.) strigose; leaflets obtuse or acute, mucronulate, rounded at base, above green, sometimes with lighter stripe along costa, sparsely strigose or strigillose or sometimes glabrous, beneath paler green, strigose or antrorse-pilose along costa and usually along chief veins, terminal one 2.5-5.5 cm. long, 0.8-2.3 cm. wide, lateral about one-third smaller; racemes several or numerous, terminal and axillary, loose below, up to 30 cm. long,

terminal one compound, axillary ones often simple; bracts purplish, hirsute-ciliate, deciduous; pedicels mostly paired, uncinately-hispidulous, erectish or somewhat spreading in fruit, 6–12 mm. long; calyx long-hirsute on lowest lobe, sparsely pubescent elsewhere, upper lip bidentate, 4 mm. long, lower lip 6 mm. long, middle lobe much longer than lateral; corolla purple, the broad banner 13 mm. long; stipe of loment 3–5 mm. long, considerably surpassing calyx; loment 1.5–2.8 cm. long, essentially equally constricted on both margins, isthmi usually short, sometimes 1 mm. long, joints promimulous-reticulate, 3–3.8 mm. long.

MORELOS.—Mountain woods above Cuernavaca, altitude 2135 m., November 19, 1895, *Pringle* 6206 (type no. 254325, U. S. Nat. Herb.); Sierra de Tepoxtlán, altitude 2285 m., November 27, 1902, *Pringle* 9744.

MICHOACÁN.—At various localities near Morelia, altitude 1900–2800 m., 1909–1912, *Arsène* 3249, 5303, 5346, 5783, 7342, 8292, 10027.

Related to *Meibomia ghiesbreghtii* (Hemsl.) Kuntze and *M. subtilis* (Hemsl.) Kuntze, both of which are now represented in the National Herbarium by good suites of specimens. In *M. ghiesbreghtii* the loment and the lower surface of the leaves are entirely glabrous; in *M. subtilis* the pod is rather densely uncinately-puberulous; and in both species the long appressed hairs of the stem of *M. bella* are wanting. *Meibomia venusta* (Steud.) Kuntze (*Desmodium elegans* Schlecht., not DC.), also closely allied, and probably identical with *M. subtilis*, is described as showing on the stem "pilos uncinatos rectosque atque in faciebus minutissimos punctiformes," while in *M. bella* the stem is strigose and finely pulverulent, without uncinately hairs. PRINGLE's no. 6206 was distributed as *D. uncinatum* DC., his no. 9744 as *D. elegans* Schlecht.

Meibomia subsessilis (Schlecht.) Blake.—*Desmodium subsessile* Schlecht. Linnaea 12:319. 1838.

This species, apparently not recognized heretofore among recent collections, is represented by a number of specimens in the National Herbarium, of which the following may be cited:

HIDALGO.—*Pringle* 7913; *Rose* 7086, 8014.

PUEBLA.—*Purpus* 2682; *Arsène* 227, 1191, 1251, 1292, 5418, 5419, 7194, 10028.

OAXACA.—*L. C. Smith* 937; *Purpus* 3047; *Pringle* 4780; *Nelson* 1585.

It is related to *M. hartwegiana* (Hemsl.) Kuntze, and several of the specimens listed have been identified as that species. In *M. subsessilis* the leaflets are chiefly lance-ovate or ovate, tapering to the often obtuse apex, thickish, impressed-veined above, densely and softly cinereous-pilose beneath, while in *M. hartwegiana* they are chiefly elliptic, not tapering to apex, reticulate above but not impressed-veined, and less densely pubescent beneath. The following specimens in the National Herbarium agree with a flowering specimen in the

Gray Herbarium of *Hartweg* 56 from Aguas Calientes, type number of *Meibomia hartwegiana* (Hemsl.) Kuntze:

COAHUILA.—*Pringle* 13699.

DURANGO.—*Nelson* 4646, *Palmer* 663 (1896).

JALISCO.—*Rose* 2511, 3568.

HIDALGO.—*Pringle* 8245.

PUEBLA.—*Arsène* 480, 10026, 10030.

Meibomia leptomeres, sp. nov.—Perennial, erect, simple below the inflorescence; stem densely uncinat-hispidulous; stipules lance-subulate, dry, subpersistent, about 8 mm. long; leaves slender-petioled; leaflets 3, linear, conduplicate, sparsely pilose; racemes elongate, slightly comose at apex; bracts purplish, about 4.5 mm. long; pedicels about 2.5 mm. long, deflexed in fruit; flowers medium; loment of *Chalarium* type, subsessile, essentially glabrous, nearly or quite straight, joints 3-4, oval, about 3 mm. long. Fig. 9.

Stems few, about 90 cm. high, essentially herbaceous, slender, terete, purplish, leafy; stipules brownish, striate, reflexed or spreading, 6-10 mm. long, persistent or deciduous; stipels bristle-form, persistent, 3-5 mm. long; petiole (1.8-3 cm. long) and rachis (7-11 mm.) very slender, uncinat-hispidulous; petiolules 1-2 mm. long; leaflets usually conduplicate, obtuse, mucronulate, rounded at base, firm, dull green above, lighter green beneath, sparsely antrorse-pilose on both sides, prominulous-reticulate especially beneath (chief lateral veins about 12 pairs), terminal one 3.5-6.8 cm. long, 5-6 mm. wide (when unfolded), lateral 3-5.8 cm. long; racemes 4, axillary and terminal, simple or sometimes bifurcate, peduncled, up to 35 cm. long, loose at maturity, pubescent like the stem; bracts ovate, acuminate, striate, ciliate, sparsely pubescent dorsally, deciduous; pedicels solitary or paired, uncinat-puberulous, 1.5-3 mm. long; calyx minutely puberulous and sparsely hirsute, upper lip bidentate, 4 mm. long, lower lip 4.2 mm. long, its lobes acutish, middle one slightly longer than lateral; corolla purple, banner 9 mm. long; loment 1-1.5 cm. long, equally lobed on both margins, glabrous or occasionally with a few short crisped hairs on isthmi and margin, isthmi strictly central, sometimes 1 mm. long, joints prominulous-reticulate, rather plump, 3-3.5 mm. long.

JALISCO.—In the Sierra Madre, west of Bolaños, September 15-17, 1897, *Rose* 2953 (type no. 301906, U.S. Nat. Herb.).

In habit and foliage this species much resembles *Meibomia arizonica* (S. Wats.) Vail, but in that plant the joints of the loment are uncinately-puberulous and much larger.

Meibomia tenuipes, sp. nov.—Suffrutescent?, much branched; stem densely spreading- or ascending-pilosulous with somewhat crisped, not hooked, whitish hairs; stipules triangular-ovate, reflexed, persistent, about 5 mm. long; leaflets 3, ovate, antrorse-pilose; racemes panicked, loose; bracts small, deciduous or sub-persistent; pedicels 8–15 mm. long, spreading or ascending in fruit; flowers medium; loment of *Chalarium* type, long-stipitate, more or less twisted, finely puberulent on surface, on margin uncinately-hispidulous; joints 3–5, oval, about 4 mm. long, their margins irregularly inflexed. Fig. 8.

Plant 60 cm. high and more; stem slender, subterete or sub-angulate, leafy; internodes mostly 1.5–3.5 cm. long; stipules acuminate, fuscous; stipels narrow, 1.5–2 mm. long; petiole (1–4 cm. long) and rachis (6–14 mm.) pubescent like the stem; petiolules 1–3 mm. long; leaflets obtuse, mucronulate, rounded at base, submembranous, above deep green, slightly paler beneath, evenly and sometimes densely antrorse-pilose on both sides with whitish or (in old specimens) somewhat rufescent hairs, prominulously-reticulate beneath, terminal one 2–5.5 cm. long, 1.5–3.8 cm. wide, lateral about one-fifth smaller; racemes axillary and terminal, numerous, up to about 15 cm. long, axis spreading-pilose or -pilosulous and sometimes with some short uncinately hairs; bracts lance-subulate or lance-ovate, about 4 mm. long, sometimes irregularly persistent; pedicels solitary or paired, finely puberulent with mostly straight hairs; calyx puberulent, ciliolate, and sparsely pilose, upper lip bidentate, 3.5 mm. long, lower lip 4–5 mm. long, lateral lobes oblong, obtusish, middle one much longer, lance-ovate, acute; corolla purplish(?), banner 9 mm. long; stipe of loment 4–7 mm. long, greatly exceeding the withered calyx; loment nearly equally lobed on both margins, 1.7–2.8 cm. long, straight or curved; joints oval or rhombic-oval, prominulous-reticulate, greenish-white, 4–4.5 mm. long, 3–4 mm. wide.

GUATEMALA.—Between Jacaltenango and San Martín, altitude 1615–2135 m., December 24, 1895, *E. W. Nelson* 3604 (type no. 252311, U.S. Nat.

Herb.); Solalá, January 31, 1915, *E. W. D. Holway* 163; mountain sides, Antigua, altitude 1675 m., December 28, 1916, *Holway* 646.

This species apparently belongs in the group of *Meibomia intorta*,¹¹ where it is distinguished by its non-uncinate pubescence, long pedicels, and long-stipitate laments.

***Meibomia cryptopoda*, sp. nov.**—Suffrutescent?; stem glabrous, sharply trigonous, with long internodes; stipules cordate-amplexicaul, persistent, longer than the petiole; leaflets 3, linear, more than 10 times as long as wide, revolute-margined, essentially glabrous; raceme terminal, slender, simple, elongate; flowers small; ovary replicate, puberulous.

Stem slender, 60 cm. high and more (base not seen), purple, obscurely granular, with few short branches; main internodes 4.5–9 cm. long; stipules lance-ovate, 8–12 mm. long, acuminate, becoming brownish, striate, erect or reflexed; stipels subulate, persistent, 2–3.5 mm. long; petiole (3–5 mm. long) and rachis (3–8 mm.) obscurely granular; leaflets obtuse, mucronulate, obtuse at base, firm, above dull green, minutely puberulous, glabrate, beneath paler green, glabrous, prominulous-reticulate especially beneath, terminal one 4–11 cm. long, 3–6 mm. wide, lateral about one-third smaller; raceme single, peduncled, about 11.5 cm. long, loosely flowered, slightly comose at apex, axis like pedicels uncinately-puberulous; bracts ovate, 4–5 mm. long, acuminate, striate, purplish-tinged, ciliolate, deciduous; pedicels solitary or paired, erect, 5–10 mm. long; calyx hispidulous, 5 mm. long, lips subequal, upper bidenticulate, lower 3-lobed, lobes oblong-ovate, obtusish, ciliolate, middle one slightly longest; corolla purplish, about 6 mm. long; ovary of *Chalarium* type, replicate, 3-jointed, puberulous with straight several-celled hairs, stipe 2 mm. long, equaling tube of calyx, isthmi central, joints oval, about 2 mm. long.

JALISCO.—Mountains near Talpa, altitude 1340–1525 m., March 7, 1897, *E. W. Nelson* 4027 (type no. 327057, U.S. Nat. Herb.).

As the ovary shows, this is a member of the group of species with replicate pods typified by *Meibomia plicata* (Schlecht. & Cham.) Kuntze. It is nearest *M. cordistipula* (Hemsl.) Kuntze, represented in the National Herbarium by

¹¹ *Meibomia intorta* (Mill.) Blake.—*Hedysarum intortum* Mill. Gardn. Dict. ed. 8, no. 11. 1768; *Desmodium uncinatum* (Jacq. 1798) DC.—See FAWCETT and RENDLE, Fl. Jam. 4:34, 1920, for synonymy.

Pringle 13421, from Tarasón, Michoacán, but differs in its very short-petioled leaves with linear, strongly revolute-margined, and much longer leaflets.

***Meibomia urarioides*, sp. nov.**—Herbaceous?; stem sharply several-angled, glabrous; stipules small, persistent; leaves slender-petioled; leaflets 3, ovate, obtuse, sparsely strigillose on both sides, thin; racemes paniced; bracts small, deciduous; pedicels about 1.2 cm. long, wide-spreading in fruit; flowers small; loment of *Chalarium* type, stipitate, replicate, glabrous, the 3-5 joints suborbicular, about 3.5 mm. long. Fig. 5.

Stem slender, 45 cm. long and more (base not seen), green, branching in inflorescence; internodes 3-13.5 cm. long; stipules subulate from a broad base, reflexed, brownish, 3 mm. long; stipels bristleform, 2 mm. long; petiole (2-3.5 cm. long) and rachis (5-8 mm.) slender, glabrous; petiolules about 1.5 mm. long; leaflets mucronulate, broadly rounded at base, nearly equally green on both sides, weakly prominulous-reticulate, terminal one 3-4.3 cm. long, 1.2-2.2 cm. wide, lateral about one-third smaller; racemes axillary and terminal, forming a loose panicle about 25 cm. long, the somewhat zigzag axes and pedicels hispidulous with spreading, curved, scarcely uncinat hairs; bracts ovate, acuminate, 2 mm. long; pedicels mostly paired, 1-1.5 cm. long; calyx hirsute, particularly on lowest lobe, upper lip bidentate, 2.8 mm. long, lower lip 3.2 mm. long, lobes ovate, acute or obtuse, lowest longest; corolla purplish, about 5 mm. long, not well seen; stipe of loment 2-3 mm. long, equaling or surpassing the withered calyx; loment 7-9 mm. long, equally constricted on both margins, closely replicate, isthmi short, central, joints thin-walled, greenish-white, prominulous-reticulate, glabrous or with a few minute hairs.

JALISCO.—Roadside between San Sebastian and Mount Bufo de Mascota, altitude 1370-2285 m., March 20, 1897, *E. W. Nelson* 4100 (type no. 327028, U.S. Nat. Herb.).

A member of the *Meibomia plicata* group, distinguished from its nearest ally, *Meibomia micheli* Blake (*Desmodium madrense* Micheli, Mém. Soc. Phys. Hist. Nat. Genève 34:258. pl. 10. 1903; not *D. madrense* Hemsl. 1880) by its strongly angled stem, persistent stipules, longer petioles, and hirsute calyx.

***Meibomia dasyacra*, sp. nov.**—Shrubby, branched; stem strigillose and near the nodes hispidulous, sometimes glabrate, inflores-

cence densely uncinat-hispidulous; stipules small, mostly deciduous; leaves short-petioled; leaflets 3, elliptic or oblong-elliptic, pilose-strigose above, beneath pale, evenly but not densely pilose and prominent-reticulate, at first softly flavescent-pilose on both sides; racemes paniced, elongate, scarcely comose, at apex flavescent-pilosulous; flowers small; ovary replicate, pubescent.

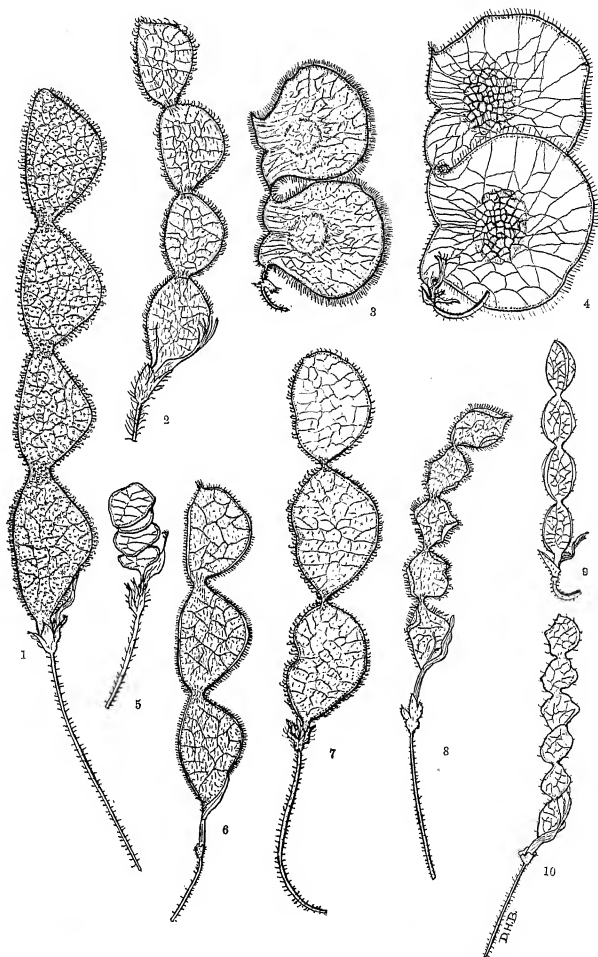
Stem slender, 45 cm. high and more, purplish; stipules subulate from a broad base, brown, firm, 3 mm. long, reflexed, rarely persistent; stipels bristleform, about 2 mm. long; petiole (0.5-1.5 cm. long) and rachis (2-10 mm.) ascending-hirsute, glabrescent; petioles 1.5 mm. long; leaflets elliptic, elliptic-oblong, or rarely elliptic-ovate, obtuse, mucronulate, rounded at base, above light green, sparsely pilose-strigose, glabrescent, subulate in age, beneath curved-pilose on the raised whitish venation (lateral veins about 5 pairs), terminal one 4-7.5 cm. long, 1.5-2.5 cm. wide, lateral one-third to one-half smaller; racemes axillary and terminal, slender, rather loose in age, peduncled, 12 cm. long or less, axis and pedicels uncinat-hispidulous; bracts lanceolate, acuminate, densely flavescent-pilose, about 2.5 mm. long, deciduous; pedicels mostly in fascicles of 2-4, spreading, 3-5 mm. long; calyx densely flavescent-pilose, lips subequal, upper bidentate, 4-4.5 mm. long, lower 3-lobed, 4.5-5 mm. long, lobes ovate-lanceolate, acuminate, middle somewhat the longest; corollas purple, about 5 mm. long; ovary of *Chalarium* type, short-stiped, densely uncinat-hispidulous on margin, antrorse- or spreading-pilose on sides, joints 3-4, oval, about 3 mm. long.

JALISCO.—Mountains near Talpa, altitude 1340-1525 m., March 7, 1897, E. W. Nelson 4028 (type no. 327058, U.S. Nat. Herb.).

A member of the *Meibomia plicata* group, nearest *M. plectocarpa* (Hemsl.) Kuntze, which is readily distinguished by its oval or suborbicular leaflets, in addition to other characters.

EXPLANATION OF PLATE IV

Fruits of *Meibomia*: 1, *Meibomia sylvestris* Blake (type), $\times 2$; 2, *M. pycnantha* Blake (type), $\times 2$; 3, *M. rhynchosma* Blake (type), $\times 1$; 4, *M. crotalum* Blake (type), $\times 1$; 5, *M. urarioides* Blake (type), $\times 2$; 6, *M. tephrophylla* Blake (type), $\times 2$; 7, *M. psilacra* Blake (type), $\times 2$; 8, *M. tenuipes* Blake (Hohway 163), $\times 2$; 9, *M. leptomeris* Blake (type), $\times 2$; 10, *M. bella* Blake (*Arsène* 5303), $\times 2$.



ANATOMY, EMBRYOLOGY, AND ECOLOGY OF ARACHIS HYPOGAEA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 325

EDWARD L. REED

(WITH PLATES V, VI, AND ELEVEN FIGURES)

The peanut, which for a number of years has been an important article of food for the peoples of tropical countries, is becoming one of the most important food and feed crops as well as a soil improvement crop in southern United States, where, on account of the ravages of the boll weevil, the agriculturist has been forced to turn to some other plant for a money crop.

Only in the last decade or two has the peanut attained the importance of an agricultural crop. Statistics from the Year-book of the Department of Agriculture show that the average annual import of almonds from 1865 to 1897 increased from 640,530 to 4,020,227 pounds; during the same period the import of filberts and walnuts increased from 3,993,921 to 12,894,106 pounds; while the import of peanuts for the same period fell from 6,522,844 to 149,672 pounds. This is explained by the increase in cultivation in the southern states. The Year-book for 1911 states: "The peanut investigations have caused a rapid extension of the industry. From a beginning of a few hundred acres in 1908, the crop this year will require more than 300,000 acres in the Gulf states alone, where prior to the time mentioned no commercial industry existed."

Before 1879 the acreage of peanuts was insignificant. From 1879 to 1889 the acreage averaged 143,000, and the next three decades show a rapid increase. The decade before 1919 shows an average acreage of 913,000, and over a million acres were planted to peanuts in each of the years 1920 and 1921. The value of the crop in 1920 was \$44,256,000. The acreage for 1921 was larger, but owing to a lower price per bushel the value was only \$33,097,000. This caused a decrease in the acreage for 1922, but it was still larger than the average for the decade preceding 1919. The value of the 1922 crop was \$29,222,000. The value of the peanut crop for the

years 1920-21-22 exceeds that of the bean for the same period. Of thirty field and orchard crops for the years 1920-21-22, seventeen had a greater acreage, one approximately the same, and twelve less than the peanut.

Papers on the peanut have appeared from time to time for the last 300 years, but they were conclusions from too few data, or mere compilations. Very little critical work has been done. Some of the early mistakes have come down through the literature and have crept into one of our best encyclopedias on agriculture and into several of the latest textbooks on southern field crops. It was for the purpose of clearing up some of these points and of making a study of its anatomy, embryology, and ecology that this study was commenced.

Literature

MARCGRAF and PISO (5) were the first to figure the whole plant. Their figure is correct except that it shows no blossoms and represents the nuts as developing on the lateral roots. WALDRON (10) divided the Linnean species into two subspecies, *A. fastigiata* for the erect or bunch type, and *A. procumbens* for the prostrate type. In his description of the types and varieties he speaks of sterile and fertile flowers; while in the description of the bunch type he mentions fertile flowers grouped at the base of the stem, and infers that sterile blossoms are found on the stems and branches higher up.

PETTIT (6) and RICHTER (7) report no root hairs, but WALDRON found them scantily near the tip under favorable conditions, and also found rosettes of root hairs at the bases of newly formed side roots. He found that these rosette hairs had the normal structure of typical root hairs, and that the root tip hairs, when present, were few, short, and scattered, occurring on young, delicate, usually few-branched roots. He found no hairs of either type on the main root. He also found that young elongating roots which bear no root hairs often have their cuticle mucilaginated, causing the soil particles to adhere as if hairs were present. WALDRON states that the mesophyll is composed of two to five-layered palisade tissue immediately below the upper epidermis, and a single layer of water storage cells next to the lower epidermis, and that these features are more typical of a xerophytic plant than is the nature of the stomata. A loose, com-

paratively thin layer of spongy mesophyll separates the palisade and the water storage layers.

In discussing the anatomy of the young gynophore, WALDRON states: "Sections of a gynophore, when very young, show a sessile ovary with usually two parietal ovules. Eleven to thirteen bundles extend from base to tip, branching more or less in their course. Along the inner edge of each bundle are tannin pockets."

Concerning the cambium layer, PETTIT states: "There is an indication of the formation of a cambium ring, although it never occurs even in the oldest portions of the organ." WALDRON considers the meristematic tissue that PETTIT noticed between the bundles to be interfascicular cambium, because he noticed xylem and phloem elements cut off from it to form secondary bundles. He considers the one-celled hairs that develop on the underground portion of the gynophore as absorptive cells simulating root hairs. WINTON (9) in discussing the pericarp, states as follows:

The pericarp or shell of the peanut, while morphologically corresponding with the pod of other legumes, exhibits some remarkable peculiarities, traceable partly at least to the conditions encountered while ripening in the soil. Not only is it deprived of chlorophyll and consequently of the photosynthetic power of the leaf, but on the other hand it is provided with root hairs, and presumably possesses to some degree the absorptive function of a true root. In other words, the pericarp, although morphologically a leaf, acts physiologically as a root. The epidermal cells are so thin-walled that they are seen with difficulty in surface view. In cross-section, especially after staining with safranin, the presence of typical root hairs arising from the center of many of the epidermal cells is evident. I have been unable to find these hairs on peanuts sold by venders, due probably to their previous removal by cleaning or by friction in the bags, but I have found them on specimens grown in North Carolina.

WALDRON describes the absorptive hairs as "large, unbranched, one-celled, and average nearly one millimeter in length." PETTIT records the cutting off of the root system of a plant, which lived several days without wilting sufficiently to be readily noticed, and suggested that this phenomenon is due to the absorption of water by the gynophore hairs, and adds, "The principal function of the gynophore hair is to furnish a supply of food material for the use of the developing fruit." RICHTER, however, states:

On the underground gynophore section the root hairs are found as a dense case on the whole outer surface. The cross-section of later sections I colored with chlorine-zinc-iodide, and found that the hairs were modified with cork, only on the surface they showed in opposition to the yellow cortex a feeble tinge in the violet of the cellulose membrane. I further treated a number of such sections with concentrated sulphuric acid and found through this reaction likewise a corking of the hairs confirmed. The hairs themselves and even the underlying two or three cork layers remained undecomposed.

Then in a footnote he adds, "Herein the result of my research stands directly opposite to that of STOCKTON-PETTIT." He further suggests that in addition to absorptive organs there is a later function of anchorage for the pod, since later "periderm is formed in the subepidermal layers that bring to an end the absorbing function of the hairs. In still later stages there appears bark structure, which gives the underground gynophore the uneven surface." PETTIT states:

Arachis hypogea is a low annual plant, with one upright flowerless branch surrounded by decumbent spreading branches, upon which flowers are borne. The stem is cylindrical and smooth, at the base becoming angular and hairy above. The stamens are monadelphous and inserted on the calyx. They are ten in number and of two kinds, one with long two-celled anthers dehiscing laterally, and one with nearly spherical one-celled anthers. In the nyctotropic movements of the leaves, . . . the main petiole sinks downward, the leaflets twist downward and backward so that the lower surfaces of each pair are applied to each other. In this position they form a little pocket shutting around the petiole, with the superior pair closed over the inferior and the tips pointed upward.

In speaking of the phenomenon of the plant's burying its seeds in the ground, WINTON says, "Shortly after blossoming the flower stalk bends downward until the young fruit is completely buried in the soil." SOLEREDER (8) states:

The hairy covering of the Papilionaceae consists of clothing and glandular hairs. The usual form of the clothing hairs is that of simple uniseriate trichomes with one or several short basal cells and a long terminal cell. Special forms of clothing hairs are as follows; . . . the multiseriate shaggy hairs of *Aeschynomene*, *Arachis*, *Chapmannia*, etc. Owing to the large number of species in which a mucilaginous epidermis has been demonstrated, usually only the genera concerned can be cited in the following list. The stomata do not belong to one and the same type (Cruciferous), as has been generally assumed (see STRASBURGER and DEBARY). The type with subsidiary cells parallel to the pore (Rubiaceous type) is also very widely distributed.

After discussing the differentiation of the mesophyll and calling special attention to the middle layer that is poor in chlorophyll and is frequently filled with tanniniferous contents, he says that in the leaves of *Chapmannia floridana* and of species of *Arachis*, *Crano-carpus*, etc., the lowest layer of the spongy parenchyma consists of cells with wide lumina and brown contents (in the dried leaf); and that according to TRECUL tannin-sacs occur in the pith in *Adesmia*, *Arachis*, and *Coronella*. He also reports that VOGELBURGER found rod-shaped crystals in species of *Aeschynomene*, *Arachis*, *Chapmannia*, etc. He says that shaggy hairs, filiform multicellular structures, have been observed only in the Hedysareae, namely, in *Aeschynomene*, *Arachis*, and *Chapmannia*.

Materials and methods

The work here reported has been done on *Arachis hypogea*. The plant as a whole has been studied in the laboratory, in the greenhouse, and in the field. Seeds have been germinated and the seedling studied as a whole. Sections have been made by the paraffin method and stained with safranin and light green, and safranin and gentian violet; and living material has been studied. The material was killed and fixed in chromo-acetic acid. Sections from the leaves of field-grown plants have been made. Flower buds, freshly opened blossoms, blossoms with withered perianth, and the lengthening gynophore in various stages have been studied. Sections were cut from 5 to 20 μ thick. The developing gynophore has been studied in field conditions, as they exist in the region of crop cultivation in Texas, and in the moist warm conditions of the greenhouse of the University of Chicago. The ecology of the plant as it grows in cultivated regions of Texas has been studied as to maximum and minimum temperature for the fruiting season, also humidity of the air, soil moisture, and wind velocity. Chemical and mechanical analyses of the soil have been made.

Classification and structure

A review of the literature shows that investigators have had various opinions as to the nativity of *Arachis*, Brazil, Africa, China, India, and even Japan having had one or more exponents as the original home. The older DE CANDOLLE (4) has ascribed it to

South America, where all the other species of the genus are found. Evidence seems to point to the fact that it was imported into Africa, from Peru, where it was taken from Brazil, and that it was carried from Africa to these countries farther east. It was introduced into the United States during the importation of slaves from Africa, being used as one of the principal articles of food for the negroes on the voyage.

Arachis has not been satisfactorily placed as to its affinities. It seems to belong in the tribe Hedysareae of the Papilionaceae. LINNAEUS placed it next to *Cicer*; PERSOON, nearer *Anthyllis*; JUSSIEU, between *Ononis* and *Anthyllis*. DE CANDOLLE, classifying it according to the character of its embryo, places it among his Geoffroyae, but at the same time, recognizing how little it conforms in other respects to these plants, suggested its forming together with *Voandzeia* a distinct tribe. BROWN says that *Arachis* and *Cercis* possess straight embryos in common with Caesalpineae and Mimoseae, in which respect they differ from the Papilionaceae.

The early botanists, before 1805, all described the structure of the blossom erroneously. The stemlike calyx tube was supposed to be a flower stalk. In 1805 PORTEAU published the first correct description of the structure of the blossom. BROWN afterward confirmed PORTEAU's description in 1816. Notwithstanding the work of PORTEAU, confirmed by BROWN, BENTHAM (2) as late as 1839 writes of *Arachis* as a plant with dimorphic flowers. He describes one form with calyx and corolla, which is always sterile, the fertile flowers having "neither calyx, corolla, nor stamens; but from two bracteolae, similar to those which are formed at the base of the sterile flowers proceeds a stiff rigid stipe or torus, which is speedily reflexed and elongated, and is terminated by what appears to the naked eye a short point. Examined under a glass this point discloses at its extremity a truncated, somewhat concave and dilated stigma."

NEISLER in 1865 found that "the flowers of *Arachis* are all petal-bearing and fertile." BENTHAM remained unconvinced, and published a reply in the same year. What BENTHAM mistook for a cleistogamous blossom was simply the elongated "peg" or gynophore after the blossom parts had fallen off. His stigma was the scar left by the falling off of the style.

Arachis is a low annual with one upright central stem, and numerous lateral, more or less decumbent branches. The central stem, as well as all branches, produces flowers and fruit. Field grown plants, late in the season, not only produce flowers in the leaf axils on the main central stem, but also in the axils of all the branches of this stem. It is not uncommon to find gynophores extending downward from leaf axils as high as 8 or 10 inches from the ground, and a number of peanuts are produced on the main stem in the lower leaf axils. The distinction between the bunch type and the prostrate type is not so clear as the names indicate. It is true that in the prostrate type all branches except the central one are prostrate from the beginning, while in the bunch type the lateral branches at first stand more or less erect; but during the latter part of the season, when the blossoms are being produced abundantly, droop toward the ground and become almost as procumbent as in the prostrate type, and a large number of peanuts are produced on the gynophores from these branches.

LEAF.—The leaf is even pinnately compound, consisting of two pairs of approximately equal leaflets, on a slender petiole of moderate length, and a pair of stipules about 4 cm. long with attenuate points. The stipules are fused with the petiole for about one-half their length. As with most compound leaves, they have nyctotropic movement. The main petiole bends downward and the leaflets move upward until the two upper surfaces are applied to each other, and the two superior leaflets usually inclose the two inferior. The pulvinus instrumental in the nyctotropic movement of the petiole is about 1.5 cm. from the stem, and at the point where the stipules separate from the petiole. My observations on the nyctotropic movements of the leaves do not agree entirely with those which PETTIT records.

As will be shown later, *Arachis* has strong xerophytic tendencies, which make the leaf structure quite striking. Stomata are present on both sides of the leaf in large numbers. They are even with the surface (fig. 1), as in plants having mesophytic characters, and are of average size. It is sparingly pubescent with short hairs. In cross-section (fig. 2) it shows a well developed cuticle, a single layer of epidermal cells, a palisade consisting of two to four layers of cells with numerous intercellular air spaces, a narrow, loosely

arranged sponge tissue, a single layer of water storage cells, and a single layer of epidermal cells with a cuticle which is not so well developed as that on the upper surface. Quite regularly certain cells of the upper epidermis project inward, forming an inverted cone which extends about one-third the diameter of the leaf. These are tannin cells and appear dark brown in stained slides.

STEM.—The stem is cylindrical, hairy, sometimes becoming more or less angular with age. The leaf nodes are usually 2–5 cm. apart. In cross-section the stem shows a large pith, which, in the older portions, owing to the breaking down of the cells, has a tendency to become hollow, this being the way in which hollow stems and stem cavities in general are formed. The epidermis has crystal cells.

ROOT.—*Arachis* has a well developed tap root with numerous laterals, which extend several inches into the ground, with very few near the surface. As a rule, all have the numerous nodules so generally found on leguminous plants. This makes it a valuable plant for rotation if the crop is harvested by pasturing with hogs; but if harvested for the nuts and the hay, as is generally done, the whole plant being removed from the field, very little nitrogen is added to the soil.

The roots are scantily provided with root hairs; in fact, it is very difficult to show that hairs of any kind are formed. As stated, PETTIT and RICHTER failed to find any, but by careful manipulation WALDRON was able to show a few short, poorly developed hairs near the tips of young lateral roots, but none on the main root. He was able to show that rosettes of hairs were developed at the base of lateral roots, and that their growth was stimulated by high temperature and high humidity, and appeared on young plants whose roots grew rapidly, but did not appear later except when the plants showed a renewed growth vigor. The normally produced tip hairs appeared on very young plants whose roots grew rapidly in moist air conditions. Saturated heavy soil conditions retarded the growth of the rosette type and inhibited the appearance of the tip hairs. I have been unable to find hairs on the root tips, and but few rosettes on field grown plants, and, since the conditions under which WALDRON was able to produce them are seldom met with in the field, it is doubtful whether they are normally produced in crop cultiva-

tion, and, when produced, are so few as to be of no practical value to the plant in the absorption of food materials. When we know that the leaf has characters typical of mesophytic plants with only a few xerophytic characters, it is difficult to explain how the plant grows so well and fruits so abundantly in the driest portion of our southern summers. Cross-sections of the root show that it is typically dicotyledonous.

FLOWER.—All flowers are complete and are produced both above and below the ground, singly or in clusters of about three in the axils of the leaves. In field cultivated plants it is not uncommon to find blossoms with yellow petals 3 inches below the surface of the soil.

The calyx is synsepalous, having a tube about an inch long crowned by five irregular calyx tips. It was this long calyx tube that the early botanists mistook for a peduncle; hence their error in describing the plant.

The corolla consists of five irregular yellow petals, typically papilionaceous, inserted on the throat of the calyx tube.

The androecium consists of ten monadelphous stamens, the filaments of which are fused into a tube for about two-thirds of their length. Eight are anther bearing, the two sterile ones being fused with a neighboring filament for almost their entire length. Sometimes only nine are discernible. There are two kinds of anthers, four long two-loculed dehiscent longitudinally, and four round one-loculed. Sections of anthers show that not all of the pollen grains develop completely. The abortive pollen grains with the reduction in the number of anthers is what one would expect in a plant that has close pollination and shows progress toward true cleistogamy.

The pistil is sessile and consists of a single carpel. The style is curved upward, bearded near the tip, and terminated with a stigma. A longitudinal section shows one locule and one to five ovules with constrictions of the ovary wall between them.

Seed and seedling

An examination of a mature seed shows it to be a straight embryo, consisting of two fleshy cotyledons, a short hypocotyl, and a plumule, the latter composed of a terminal and two lateral buds, the whole inclosed within a thin testa. These three buds are well

developed when the seed is mature, and consist of two or more compound leaves. The reserve food stored within the seed consists approximately of 26 per cent protein, 38 per cent oil, and 24 per cent starch, sugar, etc.

In germinating, the hypocotyl elongates and the cotyledons are raised above the ground. The terminal bud develops more rapidly than the lateral ones, but all three grow so that from the beginning the young plant has three principal branches. This is of especial advantage to the bunch type in which such a large percentage of the nuts is produced in the lower leaf axils. The terminal branch continues in an upright position, while the lateral and all secondary branches are more or less decumbent or spreading.

Embryo sac

The embryo sac is oval, with the antipodal end tapering and bent slightly toward the chalazal side. It is filled with starch grains in such quantity that it is difficult to see the nuclei (fig. 3), which in the mature embryo sac are eight in number. The egg apparatus and antipodals are normally placed, the latter soon disintegrating so that it is difficult to find them (fig. 4). After fertilization the endosperm nucleus rapidly divides, forming a great number of free nuclei which arrange themselves against the wall. Cell walls are not immediately formed around these, except near the egg apparatus (fig. 5). The starch grains previously mentioned disappear, having been utilized as food for the developing endosperm nuclei. The embryo sac now consists of a large cavity filled with a homogeneous fluid surrounded by a layer of nuclei held together by a rather dense mass of cytoplasm. As the embryo develops, walls are formed around the remaining nuclei, thus forming a single layer of cells lining the sac, which is the only tissue formed by the endosperm nucleus. This layer of cells lying between the embryo and the wall corresponds to the single layer of endosperm cells left between the developing embryo of the sunflower seed and its wall when the other parts of the endosperm are used as food.

Development of embryo

The egg on germinating divides horizontally, forming a two-celled body just as if it were going to form a suspensor of a single layer of cells (fig. 5), but in further development the divisions are in

three planes, and a suspensor is formed having, in optical view, two rows of cells (fig. 6). At this stage it is easy to distinguish the embryo-forming region from the suspensor by its smaller cells and denser protoplasm, and, as development continues, a longer suspensor is produced, which is no larger in diameter (fig. 7), however, pushing the embryo region farther into the sac. Up to this time there is very little enlargement of the embryo portion; but, as this region develops, the suspensor enlarges, becomes sagittate, with the narrowest portion four or five cells in diameter, which gradually expands to its union with the embryo (fig. 8), and the point becomes sharp, pushing its way among the cells of the nucellus (figs. 9, 10).

The large size of the cells of the suspensor is quite noticeable when compared with those of the proembryo, and should no doubt be explained physiologically as an adaptation that aids in the rapid transfer of food from the nucellus to the embryo, since there are fewer cell walls to be traversed. The nature of the cells of the nucellus which surrounds the suspensor adds weight to this interpretation, since the suspensor is kept closely appressed to the wall of the embryo sac, the cells of which are elongated and arranged in rows more or less at right angles to its surface (figs. 6, 8, 9, 10). These serve as food conducting tissue, carrying the digested food of the nucellus and ovule wall to the suspensor.

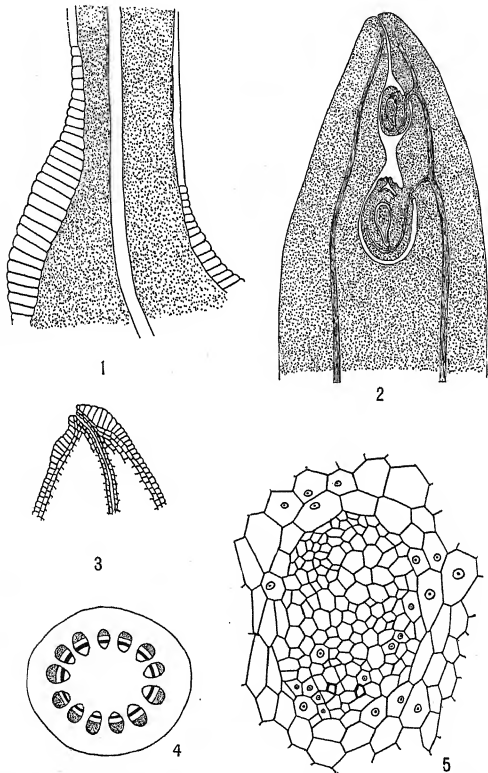
There are three different groups of cells in the ovule wall: (1) Those immediately surrounding the vascular bundles, which are small, having dense protoplasm and relatively larger nuclei than those of the other two groups, and which serve as conducting tissue, carrying transported food to the storage region. (2) The storage region, consisting of large cells in which abundance of starch grains are found. (3) The food conducting region, which lies next to the embryo sac. With the exception of the rows of elongated cells of the nucellus at the micropylar end, the food conducting region consists of one or two rows surrounding the sac (figs. 10-12). The storage region comprises by far the greater portion of the ovule wall, and the density of the reserve food increases from the outside inward, reaching its maximum near the inner wall. The two or three rows of storage cells lying next to the conducting region show but few starch grains, due to their rapid digestion and absorption (fig. 11).

The first indications of the embryo are to be seen in two swellings, the cotyledonary primordia (fig. 13), at opposite points in the proembryo, due to accelerated growth at these points. These primordia soon develop into diverging projections, between which is the stem tip initial (fig. 10). As development proceeds, the enlarging cotyledons become closely approximated, being forced together by the walls of the sac (fig. 12). Between them is the plumule, consisting of a terminal bud and two lateral ones developed in the axils of the cotyledons, thus forming a complex plumule. These buds are well developed, and are composed of two or more compound leaves; therefore, in germinating, the plumule expands into typical leaves of the adult plant. The short hypocotyl is the last to make its appearance, its tip developing at the top and a little to one side of the center of the massive suspensor (figs. 10-12). The mature seed occupies the entire space within the integument, and is a straight embryo.

Gynophore

As soon as fertilization is accomplished, the internode between the ovary and the receptacle begins to elongate, and soon turns downward at such an angle to the stem as to reach the earth in the shortest distance. The magnitude of the angle, therefore, is determined by the magnitude of the angle formed by the stem and the earth. This elongation is produced by a region of meristematic tissue lying just behind the ovary, the central region forming the pith, and the peripheral the vascular bundles and the cortex. The tip of the ovary becomes pronounced by the elongation and lignifying of a group of epidermal cells, which in their development push the base of the style to one side and form a cap analogous to a root cap (text fig. 1).

As soon as the gynophore has pierced the ground to a depth of 2-5 cm., the ovary, which up to this time has remained dormant, begins to develop, the end of the gynophore becomes swollen, and, by unequal growth on the side next to the base of the plant the ovary takes a horizontal position, so that the two or more ovules come to lie in the same straight line parallel to the surface of the earth. The lower ovule develops first, followed by the others in succession, so that, if any one fails to develop, it is usually the one in the end of the ovary.



FIGS. 1-5.—Fig. 1, tip of ovary forming cap by elongation and lignifying of epidermal cells; fig. 2, longitudinal section of gynophore showing one locule with two anatropous ovules; fig. 3, cap formed by elongated lignified epidermal cells; fig. 4, diagrammatic drawing of cross-section of gynophore; fig. 5, cross-section of very young bundle with no sign of cambium.

DARWIN (3) has shown that the growth downward of the gynophore is not due to negative phototropism, and infers that it is due to positive geotropism, and WALDRON found some granules in the lumen of the tip cells and suggests that their presence stimulates positive geotropic movement. Sometimes if the gynophore fails to reach the ground, due to the distance that the flower is from it, the ovary will begin growth and form an enlargement at the tip and then develop chlorophyll, but it never matures seed. The development of chlorophyll by an ovary that has failed to reach the ground, and by those that have been exposed to light by the removal of the soil, shows the retention of functional chloroplasts after long periods of disuse.

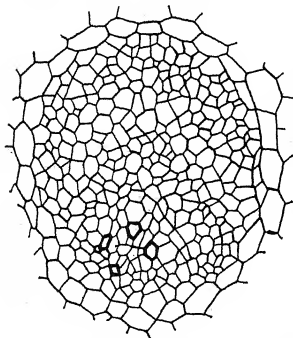


FIG. 6.—Section of older bundle with enlarged cells just beyond xylem strands, cambium initials.

A longitudinal section of the tip of a gynophore shows well developed vascular strands, one locule, and anatropous ovules (text fig. 2). The base of the style is pushed to one side by the development of elongated epidermal cells, which form a protection for the delicate tip while piercing the ground (text fig. 3). Text fig. 4 is a cross-section of a mature gynophore, showing a large pith, a vascular ring of thirteen bundles, and a

cortex. While eleven to thirteen are the usual numbers for vascular bundles, however, it is not uncommon to find as many as fifteen or more, due to the action of interfascicular cambium. A cross-section of a very young bundle shows no sign of cambium and only a few xylem strands, the whole being surrounded by large cortex and pith cells (text fig. 5). A cross-section of an older bundle shows a group of thin walled cells just beyond the xylem strands; these are the cambium initials (text fig. 6). Text fig. 7 shows mitosis in one

of the cambium initials forming the first cambium cells, while text fig. 8 is of a still older bundle in which the cambium has formed almost completely across. Text fig. 9 is of a mature bundle with well developed xylem, cambium, phloem, a large lignified bast, and interfascicular cambium.

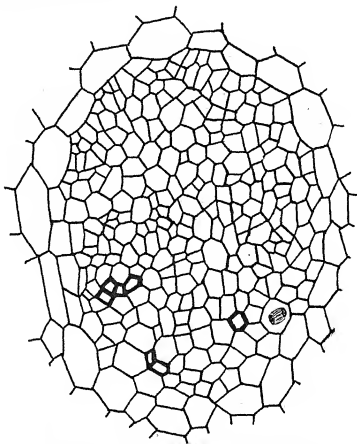


FIG. 7.—Cross-section of young bundle showing mitosis in cambium initials

Ecology

The plot studied during the season of 1923 was about one-tenth of an acre. The nuts were carefully shelled before planting, and no seed with a broken testa was used. The rows were 1 m. apart, and the seeds were planted every 15 cm. in the row. The plot, a fine sandy loam, was planted the first week of May, this being the usual time of planting in this section of Texas. In about ten days the young plants were all through the ground with practically 100 per cent germination. They grew vigorously, and soon had strong upright branches with smaller lateral branches from the axils of the cotyledons.

SOIL.—Geologically, the soil is known as Windthorst fine sandy loam, and is low in nitrogen, total phosphoric acid, and in active phosphoric acid; it is neither acid nor alkali. It is 8-12 inches deep, with a red to yellowish clay subsoil. Surface drainage is good, but drainage by percolation is poor. The types of vegetation

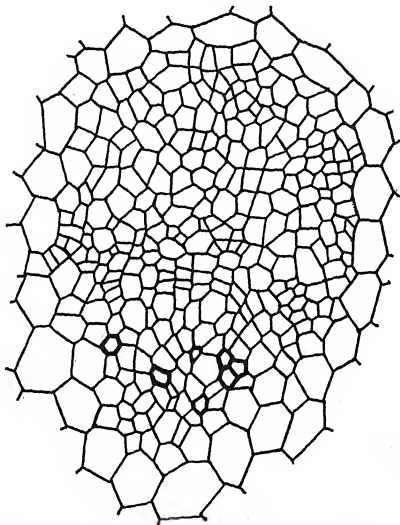


FIG. 8.—Section of bundle showing cambium almost completely across

found on unbroken Windthorst fine sandy loam in this immediate vicinity are mesophytic and xerophytic, since this is a transition zone between the mesophytic region of the eastern portion of the state and the xerophytic portion of the western plains.

RAINFALL.—The rainfall was measured by a standard rain gauge of the Weather Bureau type, located about 20 feet from the western edge of the plot. Text fig. 10 shows the rainfall from April 1 to Sep-

tember 13. Since the rainfall for April was 3.62 inches, the soil was in good condition as to moisture when the seeds were planted. The small rainfall of only 0.51 inches in May permitted the soil to dry to such an extent that truck and garden crops in the immediate vicinity suffered, but its effect upon the peanuts was scarcely noticeable. During the first thirteen days of June the rainfall

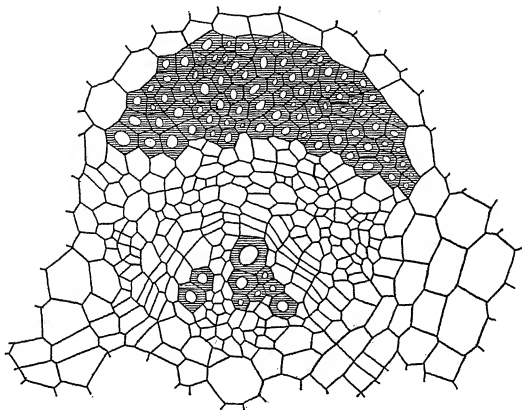


FIG. 9.—Cross-section of nearly mature bundle showing xylem, cambium, phloem, and lignified bast; cambium extends beyond limit of bundle and is, therefore, interfascicular cambium.

was 2.88 inches, and this was followed by a period of ninety-six days, ending September 14, in which the precipitation was only 2.25 inches, with the greatest single rainfall of 0.96 inches on July 1 and the second greatest of 0.80 inches on August 27. This amount of rainfall is insufficient for normal crop production, and most farm and truck crops suffered considerably, with the yield materially reduced. June corn due to fruit during this period produced from one-third to one-half crop. The peanuts on the plot and those in field cultivation on the College Farm about one and one-half miles

distant were not materially affected, although this was the period of blossoming and setting of fruit. From September 15 to September 18 inclusive there was a rainfall of 2.70 inches, and no more rain fell until after the crop was harvested on October 1, at which time there were but few immatured nuts on the vines. A few plants were left in the field and were harvested on November 30, but these

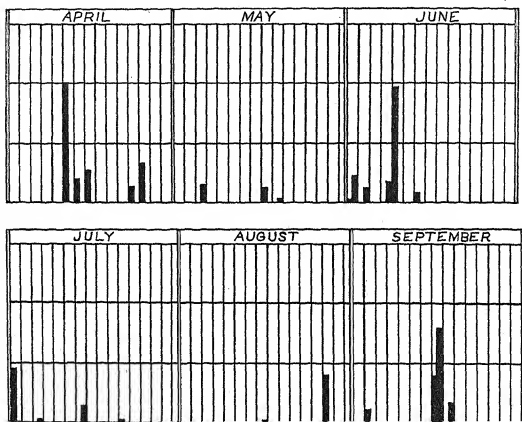


FIG. 10.—Graph of soil moisture content during fruiting period

showed no increase in number of nuts, showing that the main crop was produced during the period of light rainfall.

Table I shows the dates on which soil samples were taken, the depth in centimeters, and the percentage of water in terms of dry soil. Two depths were used: 5 cm., the depth in which the nuts are generally ripened, and 15 cm., the depth of the main root system. Text fig. 11 shows these percentages graphically. The soil samples were taken during the period in which the crop was produced, that is, from July 25 to September 15. Thus the upper 5 cm. of soil, that in which the main crop of nuts was produced, was exceedingly

deficient in moisture, and the soil in which the main body of roots grew was not abundantly supplied with water. The wilting coefficient of the soil, determined by growing peanuts, Indian corn, and tomatoes in pots, is about 2.5 per cent.

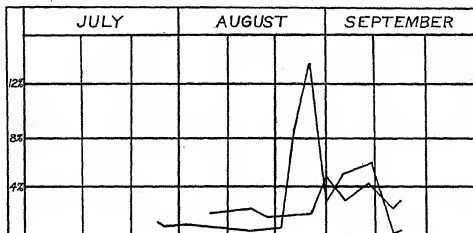


FIG. 11.—Graph of rainfall during month preceding time of planting and during growing season.

TABLE I

WATER CONTENT OF SOIL SAMPLES FROM FIELD IN WHICH PLANTS WERE GROWN

Date	Depth (cm.)	Percentage water	Date	Depth (cm.)	Percentage water
July 25.....	5	0.99	August 27.....	5	13.70
July 25.....	5	0.76	August 31.....	15	4.30
July 28.....	5	0.64	August 31.....	5	2.40
August 3.....	5	0.67	September 4...	5	5.10
August 6.....	15	1.80	September 4...	15	2.70
August 15.....	15	2.00	September 9...	5	5.90
August 15.....	5	0.48	September 9...	15	4.10
August 18.....	15	1.30	September 13...	15	2.00
August 21.....	15	1.30	September 13...	2.5	0.22
August 21.....	5	0.51	September 15...	2.5	0.77
August 23.....	5	9.90	September 15...	15	2.80
August 27.....	15	1.60			

WALDRON, WINTON, PETTIT, and RICHTER regard the hairs on that portion of the gynophore which is underground as absorptive hairs, absorbing water and food material for the plant in general, or for the developing fruit. Since the ground in which the fruit is developed has such a small percentage of water, and since it is difficult to find these hairs on normal field grown plants, it is very improbable that the peanut is affected to any great extent by the

presence of hairs on the gynophore. The plot under the climatic conditions here given produced a crop of about 20 bushels per acre, which is considered normal for such soils.

METEORIC CONDITIONS.—Table II gives the meteoric conditions during the month preceding the planting time and for the four months during which the plants grew. The number of clear days for the four months was 123, of partly cloudy 12, and of cloudy 9; hence there was a great predominance of clear weather. Table III shows the velocity and direction of the prevailing wind. For

TABLE II
METEORIC CONDITIONS DURING PERIOD OF GROWTH

Month	Number of days clear	Number of days partly cloudy	Number of days cloudy
April.....	16	9	5
May.....	27	4	0
June.....	22	3	5
July.....	28	1	2
August.....	25	4	2

TABLE III
VELOCITY AND DIRECTION OF PREVAILING WIND

Month	Average velocity per day	Average velocity per hour	Direction of prevailing wind
May.....	142.0	6.0	South
June.....	113.0	4.7	16 north 10 south
July.....	65.6	2.7	North
August 1-September 21.....	74.0	3.0	South

May the average velocity was six miles per hour, for June four and seventh-tenth miles, for July two and seven-tenth miles, and from August 1 to September 21 it was three miles per hour. During May, one-third of June, and from August 1 to September 21 the prevailing wind was from the south. Table IV shows the temperatures for the month preceding the time of planting and for the four months during which the crop grew and set fruit. From the time of planting to the time of harvest there was a gradual increase in maximum and minimum temperatures, with a corresponding decrease in range, and the high means with the velocity and direction of the prevailing wind indicate a high rate of transpiration. It is difficult to explain the ability of the peanut plant to thrive so

well with the mesophytic nature of its leaf structure in a region with the climatic conditions as they exist here.

TABLE IV
TEMPERATURES FOR GROWING AND FRUITING PERIOD

Month	Maximum	Minimum	Mean maximum	Mean minimum	Mean	Range
April.....	80	38	61.2	46.3	53.7	42
May.....	99	45	73.2	51.8	65.5	54
June.....	103	51	86.8	65.7	76.2	52
July.....	104	62	92.7	69.8	81.2	42
August 1-15.....	105	68	102.6	71.3	86.9	37

Summary

1. *Arachis hypogea* is a native of Brazil, but its wild ancestor is not known. While it is a member of the Leguminosae, it has not been definitely placed as to its affinities.

2. It is a low annual which, by a lengthening of the internode just below the ovary, pushes its pod below the surface of the soil where the seeds are ripened.

3. The root system is that of the tap root type. Root hairs are of two kinds, normal root tip hairs, and rosettes at the base of the lateral roots. The hairs are so scantily developed in field grown plants that their influence as absorptive organs is negligible.

4. The seed is a straight embryo, and consists of two large cotyledons, a short hypocotyl, and a plumule composed of a terminal and two lateral buds.

5. The mature embryo sac has eight nuclei and numerous starch grains. The antipodals soon disappear. The endosperm nucleus forms numerous free nuclei, which later form a single layer of cells lining the sac.

6. The fertilized egg produces a massive proembryo, which early differentiates into the embryo region and the suspensor.

7. The gynophore has typical stem structure. There is a large pith, about thirteen vascular bundles, both fascicular and interfascicular cambium, and a cortex. It responds to positive geotropism.

8. It grows well and fruits abundantly in hot, relatively dry climates, although the leaves are typically mesophytic with a few xerophytic characters.

I am greatly indebted to Professor W. J. G. LAND, under whose direction the work was done, and to Professors H. C. COWLES and G. D. FULLER for helpful suggestions.

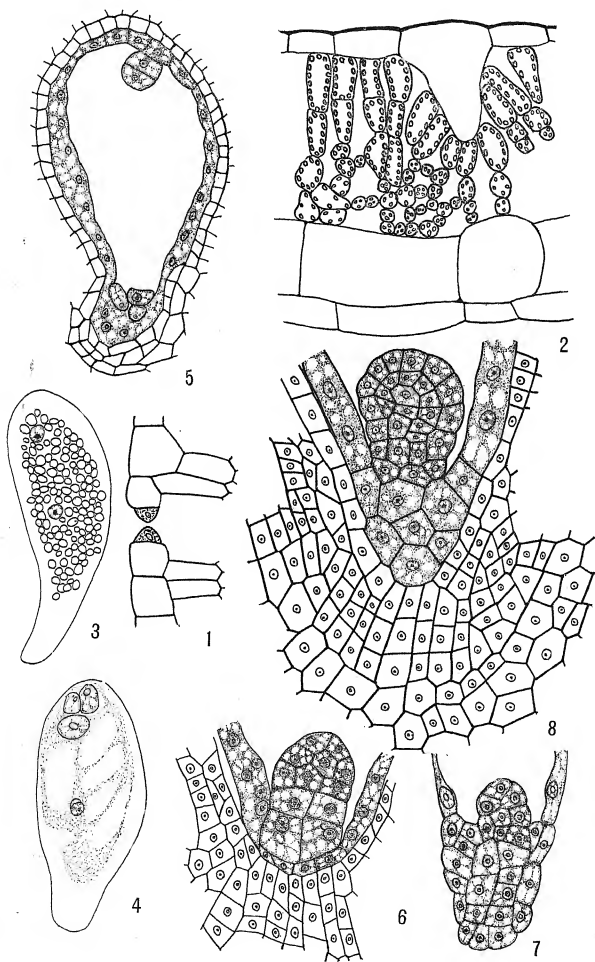
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LITERATURE CITED

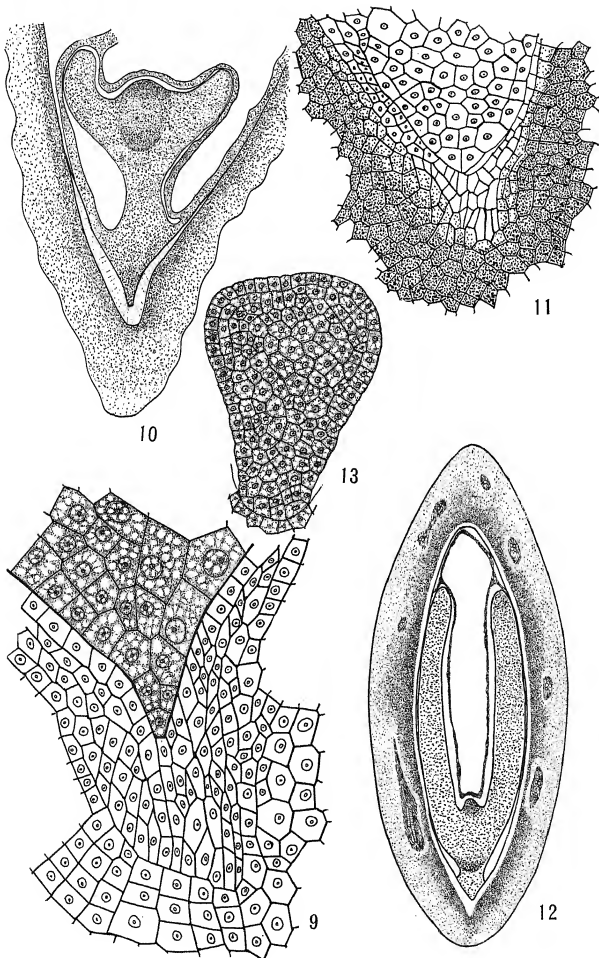
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EXPLANATION OF PLATES V, VI

- FIG. 1.—Section through stoma, showing location at surface of leaf.
- FIG. 2.—Section of leaf, showing open arrangement of palisade, small open sponge, single layer of water storage cell, tannin sac, and epidermis consisting of single layer of cells.
- FIG. 3.—Embryo sac with two nuclei and mass of starch grains.
- FIG. 4.—Embryo sac, showing egg apparatus and two polar nuclei.
- FIG. 5.—Embryo sac with free nuclei against wall; synergids at antipodal end, fertilized egg divided once, and cell walls formed around nuclei in vicinity of egg.
- FIG. 6.—Proembryo with neighboring nucellus cells; embryo-forming cells distinguished from suspensor by denser protoplasm and smaller cells.
- FIG. 7.—Older proembryo with longer suspensor.
- FIG. 8.—Still older proembryo with embryo-forming region enlarging; arrangement of nucellus cells also shown.
- FIG. 9.—Point of suspensor and its relation to food conducting region of nucellus.
- FIG. 10.—Stem tip, root tip, elongating cotyledons, and sagittate suspensor; also food conducting region at micropylar end of embryo sac.
- FIG. 11.—Foot of older suspensor than in fig. 9, with portion of storage region.
- FIG. 12.—Longitudinal section of ovule, showing three regions of ovary wall (vascular, storage, and conducting regions).
- FIG. 13.—Primordia of cotyledons.









RELATION OF LOW TEMPERATURES TO RESPIRATION AND CARBOHYDRATE CHANGES IN POTATO TUBERS¹

E. F. HOPKINS

(WITH SEVEN FIGURES)

It is well known that respiration, like other physiological processes, is greatly influenced by temperature. The literature on this subject is discussed by GORE (7), and more recently by CZAPEK (5). The general conclusion is that, for a certain temperature interval at least, respiration in plants increases with temperature according to the empirical rule of VAN'T HOFF, which was first applied to purely chemical reactions. This rule states that for every rise of 10°C . the respiratory rate is doubled or trebled.

Besides the direct effect of temperature on respiration, it is also believed by PALLADIN (12) that there is a stimulating effect caused by temperature changes. He obtained a large increase in respiration intensity in the case of bean seedlings, by exposure both to low temperature and to high, after returning them to the original temperature. Little consideration, however, has been given to the effect of continuous holding at the lower temperatures on respiratory activity, and of course this is of importance in connection with cold storage, besides having perhaps some theoretical interest.

In the case of potato tubers it was found by ZIEGENBEIN (16) that as the temperature was lowered from 45°C . to 10°C ., the respiration fell to a very low value. It is generally considered that the respiration at 0°C . is practically nil (1). In order to gain more information concerning physiological activities at temperatures below 10°C ., a somewhat detailed study has been made.

Methods

The apparatus described by GORE (7), somewhat modified, was used, a large desiccator serving as the respiratory chamber.

¹ Contribution from the Marble Laboratory. The writer wishes to express his thanks to Mr. L. M. MARBLE for his interest in the work, and to Dr. LEWIS KNUDSON for reading the manuscript.

The carbon dioxide absorption was carried out in Reiset absorption tubes, and when samples were not being collected the outlet from the desiccator was connected to the suction, so as to allow a constant current of air to flow through the apparatus. The usual precautions to assure complete absorption of the CO_2 evolved and to prevent atmospheric carbon dioxide from entering the respiratory chamber were taken. About 4-5 kg. of potatoes were used, and samples were taken for about five hours at certain intervals. The results in each case were calculated to milligrams of carbon dioxide per kilogram hour.

The carbon dioxide was estimated as follows. The gas was absorbed in 100 cc. of $\text{N}/5$ NaOH , free from carbonate, in the Reiset tube mentioned. The walls were then rinsed with several portions of distilled water, which were allowed to run into the filter flask. Titration was carried out with $\text{N}/2$ HCl in the same vessel, after adding an excess of barium chloride solution and about 40 cc. of neutral 95 per cent alcohol. Orthocresolphthalein as recommended by CLARK and LUBS (4) was used as an indicator, and very sharp end points were obtained. The addition of the alcohol increased the sharpness of the end point, especially when considerable barium carbonate was present, and this was considered to be due to the fact that the dissociation of the BaCO_3 in the presence of alcohol is very small. This method the writer believes is preferable to the double titration method using phenolphthalein and methyl orange, where the end points in both cases are uncertain and empirical. The exact strength of the NaOH solution need not be known, as the amount of CO_2 can be calculated from the HCl solution, which is easily and accurately standardized gravimetrically by means of silver chloride.

The sample titration of a solution of sodium bicarbonate shown in table I gives an idea of the accuracy for small amounts of CO_2 . Ten cc. of a weak NaHCO_3 solution was added to each of the determinations, and may be considered as equivalent to a certain amount of CO_2 . None was added to the blanks. The difference in the back titration with $\text{N}/2$ HCl between the determinations and the blanks, therefore, is equivalent to the number of milligrams of CO_2 .

as shown. Much larger amounts were determined in the actual analyses and the percentage of accuracy much greater.

TABLE I
ACCURACY OF TITRATION METHOD FOR CO₂

	BLANKS		DETERMINATIONS			
	I	II	I	II	III	IV
Back titration cc. N/2 HCl.....	41.44	41.41	40.89	40.91	40.94	40.90
Titration less blank cc.....			0.53	0.51	0.48	0.52
Equivalent to mg. CO ₂			5.8	5.6	5.3	5.7

Experiments

EXPERIMENT I.—The first experiment was carried out at two temperatures, 0° C. (32° F.) and 4.4° C. (40° F.). American Giant tubers were used, and were carefully selected as to uniformity in shape and size, and as to freedom from injuries, rots, etc. The apparatus was set up in duplicate for each temperature, in cold storage rooms where the variation in temperature amounted to about 1° C. The potatoes had previously been placed in these rooms November 23, 1922, from the cellar storage, where the temperature at the time averaged about 10° C. (50° F.). The data are summarized in table II.

TABLE II
RESPIRATION OF TUBERS AT 32° AND 40° F.

Date	11/28	11/29	12/1	12/4	12/5	12/6	12/7	12/8	12/9	12/12	12/15	12/18	12/22	1/3	1/7	1/20
Milligrams CO ₂ per kg. per hour 0° C....	5.6	2.9	4.7	5.3	5.6	4.9	5.8	6.3	6.2	7.5	6.8	4.9	4.2	3.3	3.7	3.3
Milligrams CO ₂ per kg. per hour 4.4° C....	3.5	2.0	2.3	2.3	2.7	2.5

It will be noted that the respiration at 0° C. (32° F.) is greater than at 4.4° C. (40° F.). For the time during which the two tests coincide, the average respiration at 4.4° C. is only about 60 per cent of what it is at 0° C. (32° F.). Fig. 1 shows this very clearly, and also that the respiration at these temperatures is not constant, but varies with the time until perhaps an equilibrium condition is reached. The respiration at 0° C. is seen to pass through a maxi-

num at which considerable carbon dioxide is evolved, and then to again approach its original value. This phenomenon is the opposite of what we should expect from our previous knowledge of the effect of temperature on respiration. In other words, the respiratory activity should be very weak at the freezing point of water. The anomaly, however, is perhaps explicable if we accept the hypothesis of PALLADIN (12), previously mentioned, that for every temperature change there is stimulation of respiration; and that in this case this effect is great enough to show itself above the

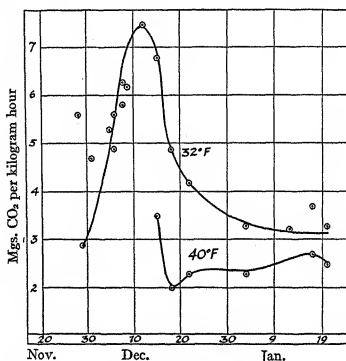


FIG. 1.—Respiration of tubers of American Giant potatoes at 32° and 40° F. plotted against time.

retarding effect of the low temperature on the purely chemical reaction. It is obvious, if these data can be verified, that, as was previously suggested by GORE (7), the Van't Hoff rule will not hold at low temperatures; but the cause would not be the injurious effect of the cold, but its stimulatory effect, and the temperature coefficient would be larger instead of smaller than calculated. The researches of MÜLLER-THURGAU (10) and others (1, 3) on the accumulation of sugar in storage organs at the expense of starch, when held at low temperatures, show that diastatic action must be stimulated at the freezing point. This is a similar phenomenon,

and therefore it appears that we are dealing with a physiological response, and not merely a chemical reaction. PFEFFER (14) has discussed this point, and states "that the action of temperature is mainly a stimulatory one, and that a change of temperature affects metabolism in a manner corresponding to the reactive power of the organism itself. . . . The physiological curve and the curve of chemical reaction hence do not coincide."

The final retarding of the respiration rate may be caused by the accumulation of certain products which inhibit the reaction. This would be analogous to the decrease of sugar accumulation in potatoes at low temperatures (APPLEMAN 1), and to the inhibition of diastatic action in fungi by the addition of cane or grape sugar, as reported by KATZ (8).

EXPERIMENT II.—In this series three kinds of tubers were used, American Giant, mature Irish Cobblers, and second crop Irish Cobblers. The respiration was determined in duplicate in each case for about five days, and the average result per kilogram per hour calculated. The temperature was then lowered five Fahren-

TABLE III
EFFECT OF TEMPERATURE ON RESPIRATION OF TUBERS OF
AMERICAN GIANT, MATURE IRISH COBBLERS, AND
SECOND CROP IRISH COBBLERS

TEMPERATURE	RESPIRATION IN MG. PER KILOGRAM PER HOUR		
	American Giant	Cobblers mature	Cobblers second crop
0° C. (32° F.)	4.22	5.04	2.83
1.67° C. (35° F.)	3.77	4.41	1.99
4.4° C. (40° F.)	3.26	5.10	2.36
7.2° C. (45° F.)	4.14	6.00	3.09
10° C. (50° F.)	5.12	8.02	3.37
12.8° C. (55° F.)	6.55	9.02	4.28

heit degrees, and after allowing a day or so for the potatoes, apparatus, etc., to come to temperature, the evolution of CO₂ was again determined.

While there may be certain objections to carrying out the experiment in this manner, the data are interesting and show at least two things: first, that there is a difference in intensity of respiration, depending on the variety and maturity of the tubers; and second,

that the value at 0°C . is greater in each case than at 1.67°C . (33°F .), and in two instances is greater than at 4.4°C . (40°F .). This latter is in keeping with the results of the previous experiment. The data are shown in table III.

EXPERIMENT III.—In order to check these results, another experiment was performed, in which the respiration of tubers of the Russett Rural variety were studied at eight different temperatures. With one exception the temperatures were maintained, as

TABLE IV

INFLUENCE OF TEMPERATURE ON RESPIRATION OF TUBERS OF RUSSETT RURAL;
IN MG. CO_2 PER KG. PER HOUR

Average temperature	3/10/23	3/12	3/14	3/16	3/19	3/21	3/23	3/26	3/28	3/31	4/2	4/4	Average
-0.83°C . (30.5°F .).....	1.0	1.6	2.1	2.0	1.7	1.8	1.8	3.0	3.0	2.8	2.1	2.0	2.07
$+0.22^{\circ}\text{C}$. (32.4°F .).....	2.4	2.8	5.2	5.7	4.8	4.4	4.2	4.1	3.1	3.2	3.99
1.17°C . (34.1°F .).....	1.8	1.8	2.3	2.8	2.5	2.3	2.3	2.1	2.1	2.3	2.4	2.4	2.25
3.2°C . (37.4°F .).....	2.5	2.3	2.8	3.0	2.1	1.8	1.9	2.2	1.0	2.0	2.1	2.1	2.14
4.4°C . (40.1°F .).....	3.0	2.8	3.4	2.6	2.3	2.3	2.2	2.4	2.3	2.1	2.2	2.0	2.47
6.72°C . (44.1°F .).....	2.8	2.0	3.5	3.1	2.8	2.3	2.2	2.7	2.7	2.8	2.7	2.6	2.76
8.67°C . (47.6°F .).....	3.2	3.2	2.4	4.8	3.6	3.6	3.1	3.3	2.9	3.34
11.5°C . (52.7°F .).....	7.7	7.0	7.8	8.6	4.7	5.7	5.4	5.4	3.7	4.7	5.3	4.5	5.87

before, in cold storage rooms, and the range of variation was about 1° . A cold water bath was utilized to obtain a temperature of 8.89°C . (48°F .). In this case the temperature varied about 1°C .

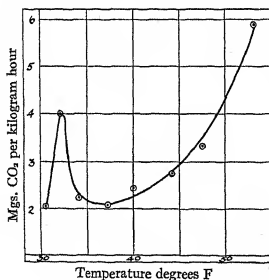


FIG. 2.—Respiration of tubers of Russett Rural potatoes at various temperatures.

The tubers were taken from cellar storage, where the temperature had been fairly constant for some time at about 4.4°C . (40°F .). The apparatus was set up in duplicate at each temperature, and the absorption and titration carried out as before. The results are presented in table IV.

The curve plotted from the average of the results over the period is shown in fig. 2. The data at any given date if plotted show a very similar type of curve, although the effect due to the acceleration at 0.22°C . (32.4°F .) is more marked for the earlier

analyses. It is evident from this curve that physiological activity, as determined by respiration, is at a low point at about 3.0°C . (37.4°F .), increases rapidly as the temperature is raised to 11.5°C . (52.7°F .), and also as it is lowered to 0°C . It declines again at -0.83°C . (30.5°F .). The complete explanation of this result is difficult; increase with increasing temperature is to be expected. The increases at 0°C . may be due to stimulation, as discussed previously, while the decrease at -0.83°C . may perhaps be due

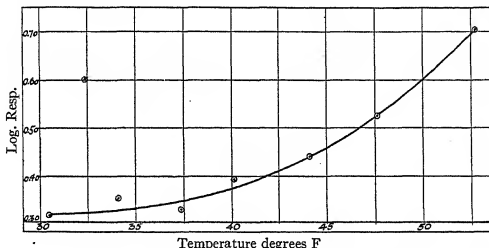


FIG. 3.—Logarithms of respiratory values plotted against temperature; Russett Rural variety.

either to the retarding effect of the low temperature on chemical reactions, or to injury of the tissue. If the logarithms of the figures representing the amounts of carbon dioxide evolved from -0.83°C .– 11.5°C . are plotted against the temperature (fig. 3), a smooth curved line is obtained if we neglect the value for 0°C . The curve appears to approach a straight line at its upper extremity.

The respiration plotted against time is shown in fig. 4 for three temperatures. Acceleration of respiration at 0°C . was again observed, while at -0.83° and 4.5°C . there is slight increase, if any.

Carbohydrate changes

The accumulation of sugar in potato tubers stored at low temperatures, and its subsequent disappearance when they are again returned to higher temperatures, has been shown by MÜLLER-THURGAU (10), and later by APPLEMAN (1). The former has suggested also that respiration is markedly affected by the changes due

to the variation in the sugar content, and BUTLER interprets his data as showing that respiratory activity of potatoes is more influenced by the amount of sugar than by temperature. It was thought that perhaps a correlation could be found between the sugar con-

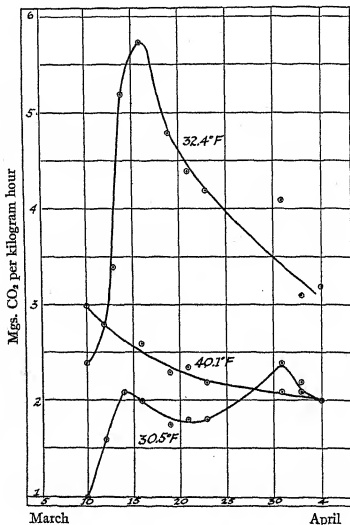


FIG. 4.—Respiration of tubers of Russett Rural potatoes at 30.5°, 32.4°, and 40.1° F., plotted against time.

centration and the respiration, which might throw some light on the mechanism by which the oxidation process is accelerated at 0° C.

METHODS OF ANALYSIS

In connection with respiration (experiment III), analyses were made of the total and reducing sugars of other tubers from the

same lot as used in the cold storage rooms, and samples were taken at certain intervals.

Sampling.—About six or eight tubers were selected, and after they were washed, several cylindrical plugs were cut from each. The cylinders were then put through a sampling machine, which reduced the tissue to a very fine pulp. The pulp was thoroughly mixed.

Moisture.—Samples were rapidly weighed in glass weighing bottles for moisture determinations, and covered with alcohol to prevent oxidation. Most of the alcohol and water was evaporated on a water bath, and then the specimens were dried to a constant weight in a vacuum over phosphorus pentoxide.

Sugar.—Fifty gm. of the pulp was weighed out to the nearest centigram, and covered with about an equal amount of 50 per cent alcohol. After extracting, making up to volume, evaporating the alcohol from an aliquot, clearing with lead acetate, and removing the excess of lead, the reducing sugars were determined gravimetrically according to the MUNSON and WALKER (9) method. After inversion with HCl in the cold for twenty-four hours, the total sugars were determined by the same method.

Hydrogen-ion concentration.—The rest of the pulp was thrown on a filter, and the filtrate allowed to drip into a beaker which was placed in a boiling water bath. This stopped the oxidase action, and also caused the filtrate to separate into a thick curd and a clear liquid. Colorimetric hydrogen-ion concentration determinations were then made on the latter, using GILLISPIE'S (6) method.

RESULTS

EXPERIMENT I.—The figures for moisture are in each case the average of duplicate determinations. The results are shown in tables V, VI, and VII.

TABLE V
CARBOHYDRATE CHANGES AT 0.22° C. (32.4° F.)

Determination	3/13/23	3/15	3/17	3/19	3/21	3/23	3/28	4/6	4/14	4/18	4/26
Moisture.....	76.45	80.28	78.34	75.86	79.40	79.00	79.48	78.94	77.27	79.74
P ₂ O ₅	6.1	5.7	6.1	6.0	6.1	6.3	6.1
Reducing sugar percentage.....	0.660	0.778	0.778	0.814	0.884	1.445	1.556	1.65	2.42	1.88	2.09
Total sugar percentage.....	1.044	1.006	1.192	1.194	1.420	1.726	1.938	2.38	2.97	3.33	3.38
Sucrose percentage..	0.365	0.216	0.459	0.361	0.509	0.267	0.353	0.366	0.465	0.427	0.272

Both the reducing and total sugar percentages are calculated on the wet basis, and expressed as percentage of invert sugar. The percentage of sucrose is obtained by multiplying the difference between the total and reducing sugar by the factor 0.9. Correction was not made for the volume of alcohol precipitate. Because of lack of time starch analyses were not made.

With one or two exceptions, the determinations of the hydrogen ion concentration in all cases are constant, within the limit of error

TABLE VI
CARBOHYDRATE CHANGES AT -0.83°C . (30.5°F .) AND 1.17°C . (34.1°F .)

Determinations	-0.83°C . (30.5°F .)						1.17°C . (34.1°F .)		
	3/19/23	3/22	3/24	3/29	4/2	4/26	3/19/23	3/23	3/29
Moisture.....	77.73	78.42	78.12	79.39	80.00	79.82	79.68	75.92	76.75
P _H	6.0	6.1	5.7	6.1	5.9	6.0	6.0
Reducing sugars percentage.....	0.638	0.660	0.710	0.486	0.158	1.83	0.828	1.112	1.244
Total sugar percentage.....	0.832	0.766	0.890	0.894	0.993	3.53	1.066	1.504	1.704
Sucrose percentage.....	0.184	0.101	0.171	0.388	0.792	1.61	0.266	0.372	0.437

TABLE VII
CARBOHYDRATE CHANGES AT 4.5°C . (40.1°F .)

Determinations	3/22/23	3/28	4/3	4/9	4/14	4/18	4/26
Moisture.....	78.04	76.36	81.69	78.72	78.87	79.97
P _H	6.0	6.0
Reducing sugar percentage.....	0.562	0.604	0.634	0.664	0.332	0.468
Total sugar percentage.....	0.760	0.674	0.756	0.768	0.652	0.650
Sucrose percentage.....	0.188	0.066	0.116	0.099	0.304	0.173

of the colorimetric method, at P_H. 6.0-6.1, and therefore this does not appear to be a factor concerned in the physiological changes observed.

The moisture content, while somewhat irregular, does not appear to vary in any particular manner. As regards reducing sugar (fig. 5), it is seen at 0.22°C . (32.4°F .) to increase slowly at first, and then rise rapidly during the thirteenth to fifteenth day of storage, after which it again increases slowly. At -0.83°C . (30.5°F .) the peculiar fact is noted that after rising to a slight maximum the percentage of reducing sugars actually decreases. At 1.17°C . (34.1°F .) for the three analyses made there is a considerable increase

in reducing sugar, while at 4.5°C . (40.1°F .) the amount is almost constant throughout, showing an increase of only 0.1 per cent in period between the first and last analysis. In almost all cases the 3 total sugars (fig. 6) parallel the reducing sugars, and are greater, showing little changes, therefore, in the sucrose content. The

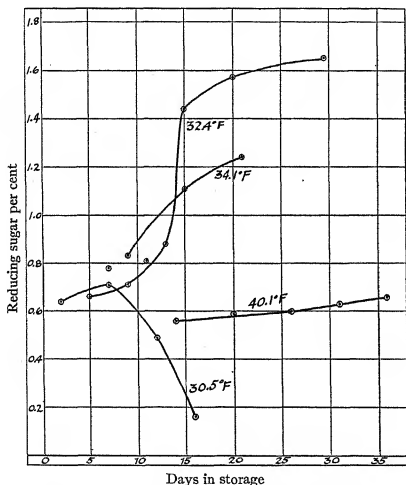


FIG. 5.—Variation in amount of reducing sugar in tubers of Russett Rural potatoes held at low temperatures.

exception to this is -0.83°C . (30.5°F .), where the total sugars gradually increase while the reducing sugars decrease. This may indicate that sucrose is increasing at the expense of invert sugar.

EXPERIMENT II.—This was performed to determine the effect of temperature change on carbohydrate transformations in the tubers used in experiment I. Tubers which had been held at 0.22°C . (32.4°F .) were moved to 4.5°C . (40.1°F .), and others

which had been held at 4.5°C . were moved to 0°C . Table VIII shows the effect on reducing and total sugar. Considerable increase

TABLE VIII

EFFECT OF CHANGING TEMPERATURE ON CARBOHYDRATE TRANSFORMATION
IN TUBERS

Date	MOVED FROM 4.5°C . (40.1°F .) TO 0°C . (32°F .)			Date	MOVED FROM 0.22°C . (32.4°F .) TO 4.5°C . (40.1°F .)		
	Reducing sugar percentage	Total sugar percentage	Sucrose percentage		Reducing sugar percentage	Total sugar percentage	Sucrose percentage
4/7.....	0.572	0.816	0.231	4/10.....	2.540	2.988	0.426
4/9.....	0.688	0.806	0.112	4/13.....	2.486	2.786	0.285
4/14.....	0.360	0.512	0.114	4/18.....	2.292	2.954	0.629
4/18.....	0.618	1.150	0.486	4/21.....	2.136	2.910	0.735
4/26.....	0.974	1.578	0.574	4/26.....	1.722	2.292	0.541

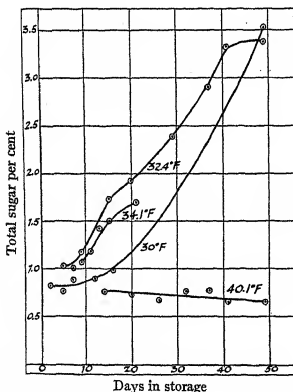


FIG. 6.—Variation in amount of total sugar in tubers of Russett Rural potatoes held at low temperatures.

(32°F .), and again declines. This increase in respiration the writer believes is due to an accelerating effect which takes place in some

in total and reducing sugar is noted in the 4.5°C . (40.1°F .) to 0°C . (32°F .) test, and a like decrease in the other (fig. 7). The latter result is in agreement with results reported in the literature on the starch-sugar equilibrium.

Discussion

From the results reported in this paper it appears that the temperature-respiration curve in the case of potato tubers does not consistently decline as the temperature decreases, but declines to a low point at about 3.0°C . (37.4°F .), increases at 0°C .

manner at or near 0°C . (32°F), which causes the respiratory activity to be abnormally high for some time.

At the time the respiration at 0°C . (32°F .) is passing through a maximum, there is a gradual accumulation of both reducing and total sugar, and when the respiration begins to decline the sugar is increasing rapidly. PALLADIN and KOMLEFF (13) have shown that the concentration of sugar in the nutrient solution in which etiolated bean seedlings were placed affects their respiration markedly, and, as before mentioned, BUTLER interprets the data of MÜLLER-THURGAU as showing that respiration in potato tubers is more influenced by the sugar concentration than by temperature. If we postulate further that there is an optimum concentration of sugar for enzyme production or action and that higher concentrations inhibit, we can explain the rise as well as the fall in the respiration rate, and also the decrease of diastatic action. This is offered as a tentative

explanation. It is possible that, as suggested by SPOEHR and MCGEE (15), increases in the amino acid content may have been responsible for the increase in the respiratory activity, but it also seems significant that the latter was accompanied by certain changes in sugar concentrations. In connection with the rest period in potatoes, such a respiratory stimulus would explain the results of MÜLLER-THURGAU (11) in breaking the rest period by holding at low temperatures, providing we accept APPLEMAN'S (2) view that the shortening of the rest period is brought about by conditions which accelerate respiration.

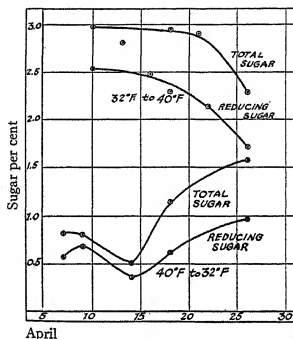


FIG 7.—Effect on sugar-starch equilibrium of moving potato tubers stored for some time at 32°F . and 40°F . to 40°F . and 32°F . respectively.

Summary

1. In determining the respiration of potatoes at low temperatures, there was found to be a marked acceleration in the rate at 0°C . (32°F .), such that for a considerable period the intensity was greater than at 4.5°C . (40°F .). This stimulation passed through a maximum and then decreased, but at the end of the experiment was still greater than 4.5°C .

2. The curve of respiration for temperatures from -0.83°C . (30.5°F .) to 11.5°C . (52.7°F .) was determined. A minimum point in this curve was found to be at about 3.0°C . (37.4°F .), and as the temperature decreases respiration increased at 0°C (32°F .), and again declined.

3. The carbohydrate changes were studied at various temperatures, and it was found that sugar accumulation at 0°C . (32°F .) is slow at first, then increases rapidly, and finally decreases. At 4.5°C . (40°F .) the sugar content is nearly constant, at 11.7°C . (34°F .) it increases fairly rapidly from the start, while at -0.83°C . (30.5°F .) there is gradual increase in total sugars but a decrease in reducing sugar.

4. In agreement with results reported in the literature, it is found that sugar accumulated in potatoes at 0°C . (32°F .) began to disappear rapidly when they were stored at 4.5°C . (40°F .).

5. It is suggested that the acceleration of respiration at 0°C . (32°F .) is dependent on the changing concentration of sugar, and that up to a certain concentration sugar increases respiration, and beyond that inhibits it.

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A STUDY IN THE GROUPING OF PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 326

HILARY STANISLAUS JURICA

(WITH FOURTEEN FIGURES)

Botanists are often impressed with the rather striking fact that frequently not only mere clumps, clusters, or groups of a given species are seen on a field trip, but at times considerable patches or even entire areas seem to be dominated by a single species. What are the factors responsible for this grouping, especially in the case of a virgin prairie? It is certainly not a felicitous scattering of seeds, for the plants are seldom annuals, and but few perennials would survive the struggle to get a foothold. At least in this case, animals, winds, or water cannot be considered as possible agencies. It is with a view to interpret this grouping that this investigation has been entered upon.

Some attention has been paid to this line of work by previous workers, notably IRMISCH, SACHS, HEGEMATER, and HOLM, and recently the subterranean organs of some of our pernicious weeds have been studied by PAMMEL and HITCHCOCK, not so much from a morphological or ecological viewpoint, but as to the means of their eradication.

On the basis of vegetative propagation, perennials have been divided into crown-formers, those that propagate by rhizomes, and those that propagate by means of adventitious buds upon creeping roots. According to HITCHCOCK (1), crown-formers are perennial plants which have no well marked adaptation for vegetative propagation, but in which the base of the stem or stems live throughout the winter, and new shoots are produced from buds upon the base of these stems. In some plants the crown is short and compact upon a well marked taproot, as in *Taraxacum*. The present investigation, however, does not cover crown-formers, but deals with both creeping horizontal and vertical rhizomes.

Horizontal rhizome

In the case of *Polygonatum multiflorum*, as described by SACHS (4), "the new lateral shoot arises each year in the axil of the ninth and last scale leaf, and the succeeding leaves are foliage leaves on slender elongated internodes, while the internodes on the basal portion between the membranous scale leaves are thick and short. The leaves are in two rows on the basal parts, alternately right and left, as may be seen by their scars."

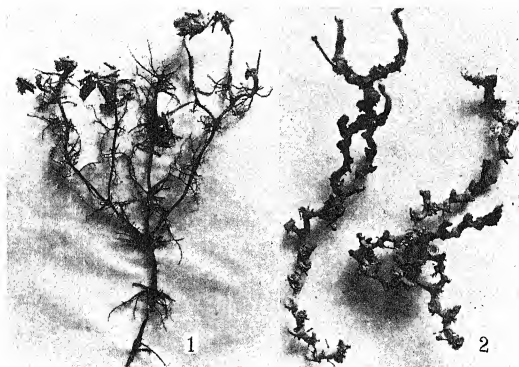
In his work on *Podophyllum peltatum*, HOLM (2) reports a similar regularity, except that at times variations occur; the bud which develops in the axil of the definite scale leaf frequently remains dormant, and another bud from another scale leaf grows out as a long horizontal shoot, continuing the direction of the rhizome. HOLM also reports that bifurcation in *P. peltatum* is not uncommon, depending upon the non-development of the regular large bud, "which is then replaced by two other buds in the axils of other scale leaves, both of which are then situated to the right and left of the axis." These two buds then develop simultaneously into two horizontal shoots with elongated internodes, only to repeat the process of producing new buds annually and new bifurcation occasionally.

To what extent is this continued, and what relation do the individual plants making up the large almost circular patch of *P. peltatum* bear to each other? HOLM, who also studied the germination of this plant, answers this in part, for he found that the fruit falls without bursting, and the seeds which are closely imbedded in the gelatinous pulp naturally germinate and grow in small clumps, flowering only after four or five years of growth. This of course accounts for the almost circular nature of the dense patches so frequently found in the woods. The extension and enlargement of the patch then depend mainly on the vegetative propagation described by HOLM, but its density in number depends upon the additional germination of new clumps within.

The writer has carefully dug up portions of a number of patches, and found that, just as regularly as the plant is proceeding in a given direction by the anterior growth and prolongation of the horizontal subterranean stem year by year, so also the horizontal rhizome is gradually and regularly subject to decay at the posterior end.

When this decay reaches a bifurcation of a probably much branched rhizome, a breaking up of this complex plant into simpler or apparently single plants is the result.

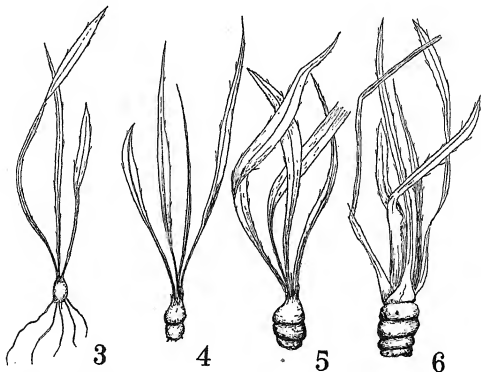
Fig. 1 shows the extensive branching of this plant, as well as its regular elongation. A counting of the scars along any one branch to the end of the main stem shows little more than seven years of growth. This is the greatest extent of time which the extensive digging revealed, due no doubt to ceasing of function and subse-



FIGS. 1, 2.—Fig. 1, branching horizontally creeping rhizome of *Podophyllum peltatum*, showing last seven years of growth and number of shoots arising therefrom; fig. 2, branched rhizome of *Smilax herbacea*, showing scars of over thirty years of growth.

quent death and decay of the posterior region. Fig. 1 also shows that this plant bears seven new shoots, and probably would have borne more if one of the branches had not been subject to mechanical injury. This means that often plants of *P. peltatum*, which from a surface view appear to be single, are in reality joined together by a common creeping rhizome. This, together with the failure of the fruit to burst and the subsequent germination of its seeds in small clumps, is responsible for the dense patches dominating an almost circular area.

That this vegetative propagation (or a modification of it) is true of many other plants with creeping rhizomes, and hence an indication of the probable cause of the dominance of a given area by a given species of a perennial, is evident from a study of *Smilax herbacea* (fig. 2), which shows the same regularity already described in producing elongated shoots in axils of definite scale leaves, the shoots alternating right and left. Unfortunately, this rhizome was damaged and parted in being dug up, but nevertheless it shows that five of the plants appearing as single are in reality produced by a



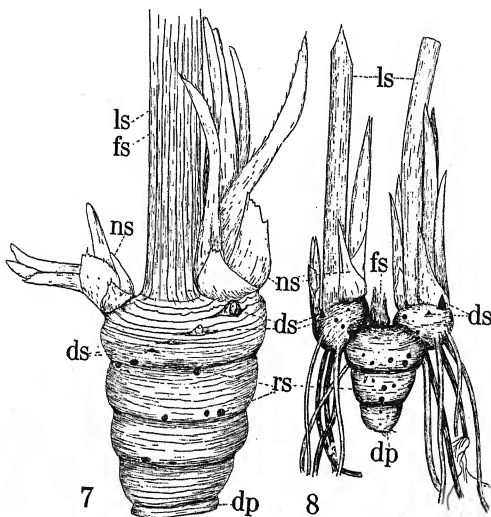
FIGS. 3-6.—*Eryngium yuccifolium*; vertical rhizome of one, two, three, and four years of growth respectively.

single branched rhizome. The woody nature of the rhizome of *S. herbacea* of course resists posterior decay more readily, hence over thirty years of growth are easily accounted for.

Vertical rhizome

What is true of plants with horizontal stems is likewise true of plants with vertical rhizomes, of course with modifications. A good example is *Eryngium yuccifolium*. The germination studies in this genus have been made by MOEBIUS (3) in his investigation upon the

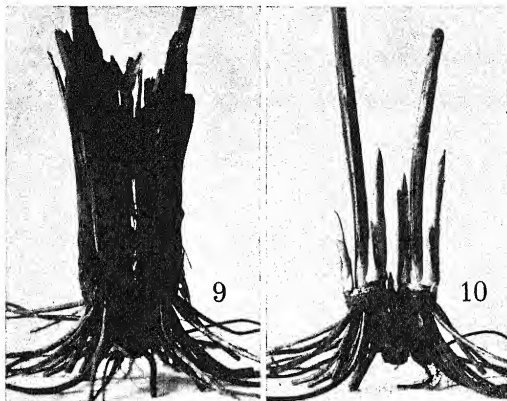
apparently monocotyledon-like nature of *Eryngium*. Unfortunately, however, MOEBIUS did not include a study of the rhizome. I found that *E. yuccifolium* does not flower until the fourth, more commonly the fifth year (figs. 3-7).



FIGS. 7, 8.—Fig. 7, *Eryngium yuccifolium*: vertical rhizome, showing five years of growth, first flowering stem, new shoots arising from axils of uppermost basal leaves, and dormant buds or shoots in axils of some other basal leaves; fig. 8, older branching rhizome; *rs*, root scars; *ds*, dormant shoot or bud; *ns*, new shoot; *ls*, last flowering shoot; *dp*, decaying posterior end of rhizome; *fs*, first flowering shoot.

Its growth is limited for the first four years, for it sends forth annually a circle of but four to seven leaves (figs. 3-6), whereas an adult flowering plant develops a circle of fifteen and sometimes even of twenty-one leaves; of course, some of these are then borne on the flowering stem. The food manufactured by the leaves for

the first four years serves to develop a strong vertical rhizome (figs. 3-7). After flowering the fifth year, as well as all subsequent years, the erect flowering stem dies, leaving a scar on the crown of the vertical rhizome. Before winter has set in, which naturally destroys the leaves and stem above the ground, a bud is produced in the axil of each of the five or six basal leaves arising from the vertical subterranean rhizome (figs. 7, 8). Only two of these buds develop



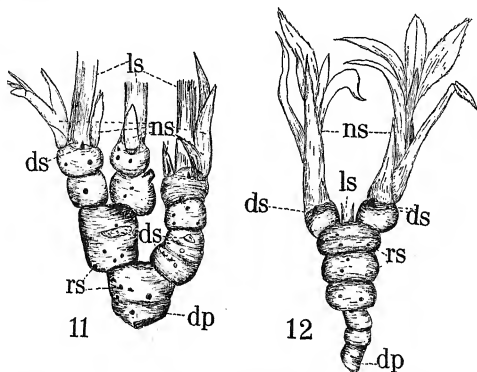
FIGS. 9, 10.—Fig. 9, *Eryngium yuccifolium*: rhizome dug up in midwinter (January 19), showing dead basal leaves protecting developing new shoots; fig. 10, basal leaves removed.

into flowering shoots; the rest abort. I have found, however, that whenever any of the ordinarily developing buds (that is the innermost and uppermost) are injured or destroyed, one of the dormant or aborted buds develops in its place. Keeping the rhizome in water for a time has also caused some of the aborted buds to begin to develop flowering stems.

As already stated, the top of the leaves above the ground freeze and dry up, but they still remain attached to the rhizome under-

ground, serving to protect the developing buds arising in their axils. Fig. 9 shows a compound plant dug up on January 19, 1921, with the old leaves attached, and fig. 10 is the same with the leaves removed. Some of the roots have been cut away so as to expose the desired feature. One can easily note the scars and old flowering stems as well as the developing buds.

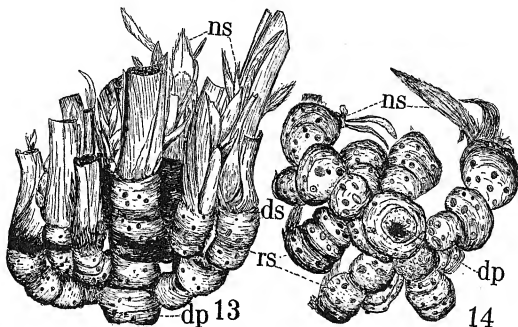
The principal feature in this study, however, is the regularity with which *E. yuccifolium* produces flowering stems, the definite



FIGS. 11, 12.—Fig. 11, *Eryngium yuccifolium*: irregular lateral branching of vertical rhizome; fig. 12, individual plant separated from group by decay of rhizome reaching region of branching.

law which it follows. It flowers the fourth or fifth year, produces five or six buds in the axils of its basal leaves, and develops two of these into flowering stems the sixth year (fig. 7). In turn these again develop buds in the axils of the basal leaves, so that after repeated years quite a group of united plants is produced (figs. 13, 14). At times one of the lateral buds fails to develop, which of course results in irregular branching (fig. 11). One could easily and fairly accurately estimate the age of any cluster of *E. yuccifolium*, were it not for the fact that unfortunately, just as in the

case of horizontal stem, decay starts in the older lower or posterior part. That is, the plant is gradually developing anteriorly, but at the same time decaying posteriorly. Whenever the decay reaches the crown of a rhizome connecting branching, superimposed



FIGS. 13, 14.—*Eryngium yuccifolium*: lateral and bottom view of much branched rhizome.

rhizomes, partition and breaking up into individual plants take place (fig. 12). To what extent this branching and subsequent grouping are possible is evident from figs. 13 and 14; hence, it is vegetative propagation which is mainly responsible for the dominance which certain species hold in given areas.

Summary

1. Many plants reproducing vegetatively spend a number of years in developing either a horizontal or vertical rhizome, before sending forth a flowering stem.
2. Both horizontal and vertical rhizomes grow at the anterior end, and die and gradually decay at the posterior end.
3. Horizontal and vertical rhizomes branch mostly by means of bifurcation.
4. New shoots arise with great regularity from the axils of certain leaves.

5. Although buds arise in the axils of a number of the leaves, but one or two develop, the rest abort or remain dormant.

6. When a regular developing shoot is destroyed, one or more of the dormant buds develop in its place.

7. Placing a rhizome of *Eryngium yuccifolium* in water causes some of the dormant buds to develop.

8. Plants reproducing vegetatively are able to invade and dominate already overgrown areas.

9. Vegetative reproduction is generally responsible for both the close grouping of many plants and their dominance in a given area.

10. The flowering shoots arising from the branches of the subterranean rhizome, viewed from the surface, give an impression of many individual plants, whereas in reality they are connected by a common rhizome.

11. When the gradual decay of the posterior end of a creeping horizontal or of a superimposed vertical branched rhizome reaches the region of branching, a breaking up of the group into non-connected individual plants takes place.

Acknowledgment is due to Professors CHARLES J. CHAMBERLAIN and HENRY C. COWLES for suggestions and criticisms.

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MEIOTIC CYTOKINESIS OF CANNABIS*

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(WITH PLATE VII)

During the summer of 1922, the writer investigated the meiotic divisions in the pollen mother cells of *Cannabis sativa*, for the purpose of throwing some light upon the cause of the variability of pollen grain size which occurs in this species. Although the results obtained were not conclusive, so far as the solution of the problems at hand were concerned, it seems desirable to publish a short account of meiosis in this plant, and to add another to the several already reported cases of cytokinesis by furrowing in the cells of higher plants. Since an excellent review of the literature dealing with this interesting phase of plant cytology has been given by FARR (2), it does not seem necessary to take space for literature review here.

STRASBURGER (6) published a few figures of meiotic divisions in *Cannabis*, mostly of the anaphase of the heterotypic division, showing ten chromosomes as the reduced number. In the root tips of the staminate and of the pistillate plants, he found that the chromosomes apparently were identical in size and shape, and no indication of a sex chromosome was observed. The results of recent research, however, have shown that so many irregularities occur in the development of the staminate buds, that a more complete account of chromosome behavior during the reduction division in this plant is desirable. The series of drawings in pl. VII show the changes which take place from the time of the archesporial cell to the liberation of the pollen grains.

Materials and methods

The material for this study consisted of staminate buds fixed whole in ALLEN'S (1) modification of Bouin's fluid. This reagent was found to be very satisfactory for fixing and preserving both the chromatic and the achromatic figures, although there is a slight tendency to cause shrinkage of the tissue. Considerable time and

* Contribution from Bussey Institution of Harvard University.

labor were saved by examining one anther from a bud while in the fresh condition, after staining and fixing with aceto-carmin. If the material proved to be in the right stage for study, the remainder of the bud was fixed at once. It was found that the best time to collect buds for studying the reduction division was between 7:00 A.M. and 11:30 A.M. Attempts to obtain satisfactory material in the late afternoon were practically without success.

The examination of large numbers of cells is facilitated by the fact that in a longitudinal section of a staminate bud many pollen mother cells are to be seen within a single loculus. In the premeiotic conditions the archesporial cells are crowded closely together, and occupy the whole space inclosed by the lining of tapetal cells. The greater part of each cell is filled with a rather dense cytoplasm, homogeneous in appearance. In the center or slightly to one side can be seen the large, nearly spherical nucleus, with a well defined membrane, and containing the deeply stained nucleolus. During the premeiotic period the chromatin is in such a fine state of division that no definite units can be observed, and it is not until the formation of chromatin threads during the early stages of synapsis that definite chromatin elements become visible.

The tapetal cells are conspicuous during all stages of meiosis as a ring of closely packed cells, lining the loculus of the anther. During the early synapsis stages of the mother cells, the nuclei of the tapetal cells divide mitotically, but the cells do not divide. These binucleate cells then remain in this condition until the rupture of the mother cell wall, when they begin to disintegrate and soon disappear.

Heterotypic division

The first indication of preparation for the heterotypic division is a slight enlargement of the nucleus and the appearance of a fine network pervading the space inclosed by the nuclear membrane. This fine mesh of threadlike elements appears to extend in all directions in a criss-cross manner, completely filling the nucleus and usually enveloping the nucleolus; but the threads are so fine and cross each other so frequently that it is impossible to make accurate counts of the number present. The next step is a gradual contraction of the chromatin material away from the nuclear membrane.

The condensation is not uniform at all points, and frequently the intertwining mass is found at one side of the nuclear cavity (fig. 4). The continued withdrawal from the periphery of the nuclear cavity causes the individual elements to become very closely massed together. The nucleolus at this time is usually found surrounded by chromatin, although cases have been observed where it was almost free and lay between the chromatin complex and the nuclear membrane. That this period of the nuclear reticulum and chromatin contraction is a relatively long one, is indicated by the fact that much of the material examined was in this stage of development, and seldom did the mother cells at the opposite ends of a locus show appreciable differences. In some of the later stages, where development is more rapid, it is possible to observe synapsis stages in one end of the locus and mother cells with two nuclei in the opposite end.

The relatively long period of contraction which passes under the name of synapsis is one during which the chromatin threads are in such close association with one another that little can be learned from observing the closely contracted mass. Fig. 5 shows the appearance of a typical cell at this time. When the chromatin complex begins to expand, the threads spread out in all directions until they reach the limiting membrane. As the threads emerge from the synaptic knot they are thicker, fewer in number, and more twisted about one another than in the early prophase. As the threads reach the membrane they contract more and more, until short, rod-shaped chromosomes are formed. The paired chromosomes which result from this contraction are shown in the region of the nuclear membrane in fig. 8.

From the end of the synaptic period, when the chromosomes are found in the region of the nuclear membrane, to the time when they are arranged in the center of the cell preparatory to being pulled apart to the opposite poles, is a period of many and rapid changes in the cell. The nucleolus, which up to this time has been very conspicuous, begins to fade and finally disappears. At about the same time the nuclear membrane very suddenly disappears. The chromosomes migrate to the center of the cell, the spindle fibers radiate into this mass from the poles of the bipolar cell, and the

chromatin units again become indeterminate. The daughter chromosomes remain in this position only a short time before they begin to move apart to the opposite poles of the cell. A polar view of the chromosomes just after they have left the plate shows them well separated and ten in number. The unusual tangential view shown in fig. 12 shows the whole twenty chromosomes as they are leaving the plate, and gives a good demonstration of their morphological identity. As the two groups of daughter chromosomes approach the opposite poles of the cell each begins to contract, and passes through a series of obscure changes, the net result of which is the formation of a complete nucleus. The heterotypic division is now complete, and the binucleate cell awaits the beginning of the homotypic division, which starts almost immediately.

Homotypic division

The homotypic division is characterized by greater rapidity than the heterotypic division, and therefore is less easily observed. At the beginning of this period the chromatin is usually found in a very fine state of division, although it is not uncommon to see small pieces of chromatin which have not undergone complete transformation in the region of the nuclear membrane. The general course of events is much the same as in the heterotypic division, but from the interkinesis condition shown in fig. 14 to the formation of the spindles is an exceedingly obscure period. The threadlike elements of each nucleus contract to form masses of chromosomes, which become arranged in the center of the nuclear cavity; the nuclear membranes and the nucleoli disappear, and spindle fibers appear. The details of these events are difficult to observe. There seems to be much less separation of the chromatin elements during this division than during the heterotypic, and as a result it is practically impossible to follow individual chromosomes. Sometimes both nuclei divide at about the same time, but in other cases one nucleus may be seen in the telophase when the other is just starting to form the mitotic figure. The divisions may both occur in the same plane, or at right angles to each other, or in various planes at different angles to one another. Fig. 15 shows both division figures parallel and in the same plane. The usual result of such an oc-

currence is that all four nuclei can be seen in one section of the mother cell, a rather unusual condition in *Cannabis*. The most usual condition is to see three of the four nuclei (fig. 17). As the chromosomes leave the equatorial plate in the homotypic division they are much massed together, and only in exceptional preparations can the chromosome number be determined. The final stages which follow are much the same as in the heterotypic division; the four chromosome groups become rounded off, a membrane forms about each, the spindle fibers disappear, and there results a cell with four nuclei. The cytoplasm is homogeneous in appearance throughout the cell, no connecting fibers are to be seen between the nuclei, and traces of a cell plate are entirely absent. The condition of the mother cell at this stage is represented in fig. 17.

Cytokinesis

After the meiotic divisions have been completed there is a relatively long period of apparent inactivity within the mother cell. Sections of whole buds frequently show all of the mother cells in this condition, with no indications of further development. The first sign of further change is the appearance of constrictions in the cytoplasm, at four points on the periphery, but inside the mother cell wall and at equal distances from the nuclei. The first visible effect of these constrictions, as seen in a section of the cell, is a change from the nearly spherical to a triangular form. The constrictions become very sharp and extend to the center of the mother cell, cutting it into four parts. Many mother cells can be found which show the completed process, but due to its extreme rapidity, cells which show the constrictions only part way to the center are more rare (fig. 19). The writer has observed a similar process in the mother cells of *Nicotiana*, where it is much less rapid in its development and correspondingly easier to follow.

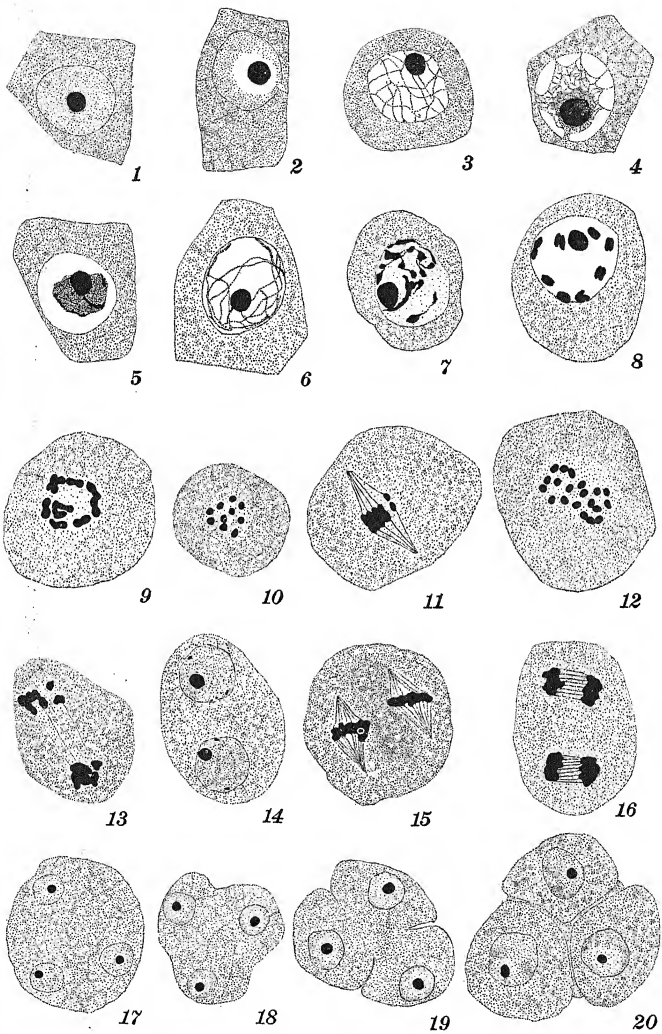
Until recent years the method of cytokinesis in the dicotyledonous plants was supposed to be by the formation of a cell plate, and this feature was one which constituted a distinction between plant and animal cells. FARR (2, 3), however, has shown that cytokinesis by furrowing occurs in the dicotyledons *Nicotiana*, *Primula*, *Helianthus*, *Ambrosia*, *Tropaeolum*, *Chrysanthemum*, *Magnolia*, and

in the monocotyledons *Sisyrinchium* and *Nelumbo*. Mrs. W. K. FARR (4) has found the same process in *Cobaea*. GATES and REES (5) reported a similar process occurring in the pollen mother cells of *Lactuca*. It is evident, therefore, that the occurrence of cytokinesis by furrowing is not as rare among plants as it was thought to be a few years ago, and it is probable that more examples will be found as more plants are investigated. FARR (3) reports that the division of the mother cells in *Nelumbo* is accomplished in part by furrowing and in part by the formation of a cell plate. A cell plate starts to form at the periphery of the cell, and after extending in for a short distance disappears. The process is then completed by the formation of furrows which divide the cell into four parts.

After the formation of the pollen grains as a result of the rapid extension of the furrows to the center of the mother cell, each grows somewhat and rounds off, although the shape of the pollen grains is never exactly spherical while they are retained within the mother cell wall. Finally, the wall of the mother cell is ruptured, the pollen grains pass through a period of growth and assume a spherical form, filling the loculus and awaiting the dehiscence of the anther.

Summary

1. Counts made in the pollen mother cells of *Cannabis sativa* show that the reduced number of chromosomes in this species is ten.
2. The reduction division commonly occurs during the period from sunrise to noon. Although mitotic figures can be found in material collected during the afternoon or evening, they are comparatively rare.
3. The heterotypic division is easily studied, and chromosome counts are most easily made during the anaphase of this division. The behavior of the chromosomes is normal, and no unusual features were noted.
4. Little can be learned concerning the individual chromosomes during the homotypic division, because of the massing of cell elements and the rapidity of the division.
5. There is no morphological evidence that any one of the ten chromosomes is a sex chromosome.



6. The method of cytokinesis is by furrowing. The furrows appear at the periphery of the cytoplasm and at points equidistant from the mother cell nuclei. These furrows extend rapidly to the center of the mother cell, cutting it into four parts.

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EXPLANATION OF PLATE VII

All drawings were made with the aid of a camera lucida, and all figures were drawn from single sections; magnification 1800 diameters.

FIG. 1.—Archesporial cell.

FIG. 2.—Archesporial cell showing shrinkage of cytoplasm.

FIG. 3.—Parasynapsis.

FIG. 4.—Contraction of chromatin.

FIG. 5.—Synizesis.

FIG. 6.—"Open spireme" showing conjugation of threads.

FIG. 7.—Condensation of threads to form chromosomes.

FIG. 8.—Diakinesis.

FIG. 9.—Metaphase of heterotypic division, polar view.

FIG. 10.—Anaphase, polar view, showing ten chromosomes.

FIG. 11.—Metaphase, side view.

FIG. 12.—Tangential view, late metaphase.

FIG. 13.—Daughter chromosomes at poles.

FIG. 14.—Interkinesis, nuclei in resting condition.

FIG. 15.—Metaphase of homotypic division, side view.

FIG. 16.—Anaphase of homotypic division, side view.

FIG. 17.—Mother cell showing three resting nuclei.

FIG. 18.—Mother cell showing beginning of furrowing.

FIG. 19.—Constrictions extending to center of cell.

FIG. 20.—Four pollen grains before rupture of mother cell wall; mother cell wall not shown.

A SPARGANIUM FROM THE MIDDLE EOCENE OF WYOMING

EDWARD W. BERRY

(WITH SEVEN FIGURES)

In studying a collection of fossil plants from the Wind River basin, of Middle Eocene age, made by Mr. N. H. BROWN of Lander, Wyoming, I have found, among other interesting forms that shed much light on the environment of the mammalian fauna known from this horizon, a series of specimens that so admirably illustrate the methods of paleobotany in dealing with fossil plant remains preserved as impressions, that they seemed to me to merit special comment.

Paleobotanists are often criticized by botanists, who insist on the futility of attempting to determine species from the foliage alone. In this connection attention is called to the fact that in general foliar organs are more conservative than either vascular, floral, or carpological structures.

In dealing with impressions of plants it often becomes necessary to describe detached objects of various kinds. Most often these are leaves, but at times are stems, fruits, flowers, and various other detached parts. Often their botanical relations are only partially understood, or they may even be entirely misunderstood. It is desirable to have such objects described and figured, however, not only because they may be of great chronological value to the geologist, but also because they are frequently of importance in the attempt to determine past environments.

It is quite possible to get a considerable knowledge of such an environment from a representative collection of fossil leaves, even though all of them are wrongly identified or even remain undetermined. This is also justified from a purely botanical point of view, since sooner or later someone with better material will obtain the clue to their true botanical position, or will be able to correlate the scattered parts into a complete and convincing whole, as I

believe that I have done for the species of *Sparganium* which is the subject of this note.

In 1882 NEWBERRY¹ described some objects from the Green River beds of Wyoming which he called *Brasenia*(?) *antiqua*. It should be noted that these objects are entirely different from the Canadian *Brasenia antiqua* of DAWSON, which latter have since been referred to *Nelumbo*. The Middle Eocene remains that NEWBERRY thought represented an extinct species of *Brasenia* consisted of branched stems bearing spheroidal fruit heads, made up of individuals which he described as small club-shaped pods, although he states that the specimens were too imperfectly preserved to permit any decisiveness in their identification. No additional material similar to that studied by NEWBERRY, which is preserved in the National Museum (no. 7018), has been collected from the Green River beds.

In 1923 KNOWLTON² published a revision of the flora of the Green River formation, describing certain leaves under the name of *Pontederites hesperia*, and these he compared with those of the existing pickerel weed, *Pontederia*. He believed that these leaves represented some Eocene member of Pontederiaceae. They lacked their basal portions, but were of considerable size, with mostly parallel longitudinal veins connected by transverse veinlets.

The Wind River material which furnished the collections for this study was contained in beds which have a high content of volcanic ash, and were for the most part water-laid, that is, they are tuffs. The surfaces of some of the slabs are covered with numerous fruit heads, dissociated fruits, and stem fragments which are identical with those from the Green River beds that NEWBERRY called *Brasenia*(?) *antiqua*. In several instances these fruit heads are attached to the branches upon which they were borne in life. These heads are of various sizes, ranging from 5 to 12 mm. in diameter, and are made up of numerous small symmetrical beaked fruits, arranged radially on a spherical central receptacle. In some cases these tiny fruits have been replaced by silica, and hence show their

¹ NEWBERRY, J. S., U.S. Nat. Mus. Proc. 5:514. 1882; U.S. Geol. Survey Mon. 35:93. pl. 68. fig. 7. 1898.

² KNOWLTON, F. H., U.S. Geol. Survey Prof. Paper 131:154. pl. 36. fig. 6. 1923.

general form with great fidelity. They are round and approximately symmetrical in cross-section, about four times as long as their maximum diameter, narrowed like the neck of a waterbottle above the middle, and slightly expanded at their distal end. Their surface is smooth, and they show no structural details. Those that are partly eroded are seen to be hollow.

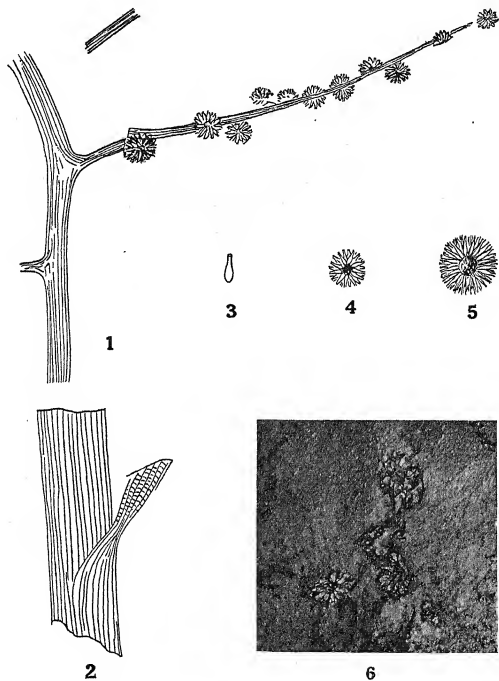
There can be no doubt but that these objects are the same as those from the Green River beds which NEWBERRY thought represented *Brasenia*, for in those Wind River specimens where the details are indifferently preserved the two cannot be distinguished, and the supporting stems and branches also are identical. The better preserved of these Wind River specimens show that they cannot represent *Brasenia*, since in that genus the fruiting heads are made up of a much smaller number of unsymmetrical carpels. The only modern forms known to the writer which have fruit heads like the fossil are those of *Sparganium*, and in this genus the individual fruits are also very like those of the fossil, consisting of numerous one-celled, nutlike fruits.

Associated with these *Sparganium*-like fruits are leaves of *Pontederites hesperia* Knowlton. These are neither common nor well preserved, but they show the unmistakable parallel veining with the transverse nervilles of that form, and they are too wide and otherwise dissimilar to be confused with the associated fragments of palm foliage, nor are they at all similar to the associated fragments of the problematical monocotyledon which has been called *Musophyllum*.

In one specimen a considerable fragment of a parallel veined stem, about 1.25 cm. in diameter, as flattened in the rocks, bears a fragment of a leaf with parallel veins and transverse veinlets exactly like *Pontederites hesperia*. This shows that the leaf was not petiolate nor auriculate at the base, but cuneate-lanceolate, narrowing at the base and then expanding to form a decurrent sheath, and that the leaves were alternate in arrangement.

This leaf-bearing stem fragment is marked by parallel veins exactly like the somewhat smaller branched stems which bear the fruit heads, and is identical with them in every respect, so that the conclusion is inevitable that both represent the same plant. This

conclusion receives corroboration, if such is deemed necessary, by their close association in the Wind River basin and by the presence of identical detached leaf fragments and fruit heads in the



FIGS. 1-6.—Fig. 1, specimen showing part of inflorescence with attached fruit heads; fig. 2, part of large stem with part of attached leaf; fig. 3, single fruit; fig. 4, small fruit head; fig. 5, large fruit head; fig. 6, photograph of several fruit heads; all figures natural size except fig. 6, which is slightly enlarged.

Green River formation, none of these classes of remains being found singly at any other horizons.

Summarizing the argument, it may be noted that fragments of leaves identical with those named *Pontederites hesperia* are found attached to particular stems; identical stems have attached to their lateral branches fruit heads like those named *Brasenia* (?) *antiqua*.

The family Pontederiaceae has petiolate, auriculate leaves, and the fruit is a single seeded utricle or a capsule; hence the fossil cannot be referred to that family. The family Alismaceae, which has leaves with a somewhat similar venation, has these leaves petiolate and frequently auriculate, and the fruits are conspicuously different, being achenes with markedly curved seeds. The only family that appears to fulfil the requirements of the fossils, so far as the latter are known, is the Sparganiaceae.

It is true that the leaflike spathes of the modern species have not been seen in the fossil material, a matter of lack of preservation of a minor detail, but in other respects the resemblance is most exact as to stem, inflorescence, fruit heads, and individual fruits. If the fossil leaves were narrowed and elongated, a most easily accomplished modification, they would be exactly like those of the modern species. I therefore propose that these fossils be referred to a single botanical species of *Sparganium*, of which I have attempted the restoration (fig. 7), and that this species be called

Sparganium antiquum (Newberry).—Plants of considerable size, at least 40 cm. tall, with stout, erect, parallel veined stems, with a few alternate sheathing leaves. Leaves broadly lanceolate, with numerous, essentially parallel, longitudinal veins, becoming crowded toward the base where they unite to form a not very conspicuous midvein, which, traced distad, becomes indistinguishable from the other veins in the upper half of the leaf. These veins are conspicuously connected by transverse veinlets which die out in the leaf sheaths, which later merge proximad with the stems, from which they are indistinguishable in appearance. The inflorescence is large and branched, fragments showing three lateral branches are preserved. These branches are at nearly right angles to the stem, rather stiff and curved, and bear from eight to ten nearly sessile

heads at somewhat irregular intervals, and progressively diminishing in size outward. It is not possible to determine whether or not the distal heads were staminate, as in the recent species of *Sparganium*, since the details of organization are not uniformly well preserved.

The material upon which this species is based is associated with an extensive warm temperate flora of a humid environment, and comes from Tipperary, Fremont County, Wyoming.

The family Sparganiaceae comprises the single genus *Sparganium* with ten or a dozen existing species, widely distributed in temperate and cool regions, growing in wet, or in some cases, submerged situations. They are chiefly confined to the Arctogaic realm, but occur

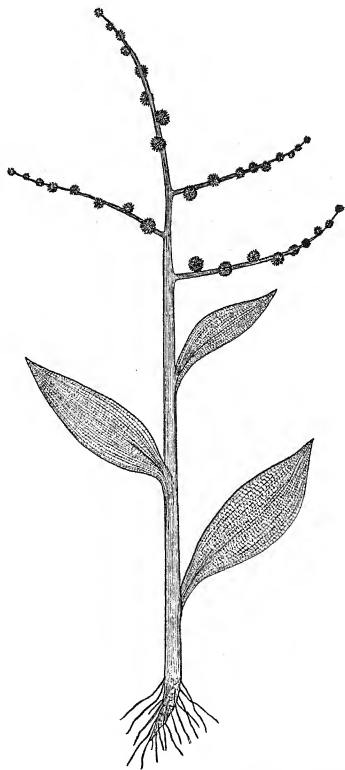


FIG. 7.—Restoration of *Sparganium antiquum* (Newberry); about $\frac{1}{3}$ natural size.

also in Australia and New Zealand. The five or six North American species have a collective range from Newfoundland to British Columbia, and southward to Florida, Louisiana, and California. The discontinuous distribution of the genus in existing floras indicates a considerable antiquity, and this is fully borne out by the geological record.

Fossil remains that have been referred to *Sparganium* are fairly numerous in the rocks. Disregarding in the present connection the presence of several existing species in the Pleistocene deposits of both Europe and America, there are about fifteen extinct species that have been referred to this genus. The oldest of these are some not very convincing fruit heads from the Upper Cretaceous Atane beds of western Greenland,³ and similar inconclusive remains from the Vermejo formation of Colorado.⁴ The Eocene has furnished one or two species in addition to the one described in the present contribution. These occur in the early Eocene Fort Union formation of Montana,⁵ and in the late Eocene, or possibly Oligocene of western Greenland,⁶ Spitzbergen,⁷ and Iceland.⁸ The genus makes its appearance in Europe during Oligocene times, when it is reported to the extent of five species in beds of that age in France, Italy, Germany, and Alsace. Seven Miocene species have been recorded from the following countries: Switzerland, France, Syria, Croatia, Bohemia, Baden, and Transylvania. The Pliocene records comprise those of three species in Italy, France, Germany, and Holland.

³ HEER, O., Fl. Foss. Arct. 3:105. pl. 28. fig. 12. 1874.

⁴ KNOWLTON, F. H., U.S. Geol. Survey Prof. Paper 101:253. pl. 32. fig. 6. 1918.

⁵ WARD, L. F., *Idem*, Bull. 37:18. pl. 3. figs. 6, 7. 1887.

⁶ HEER, O., Fl. Foss. Arct. 1:97. pl. 45. fig. 2. 1868: *Idem*, 2:467. pl. 42. figs. 4, 5. 1871.

⁷ ———, *Idem*, 2:51. pl. 7. fig. 3c. 1870.

⁸ ———, *Idem*, 1:145. pl. 25. fig. 1. 1868.

CLADOSPORIUM FULVUM

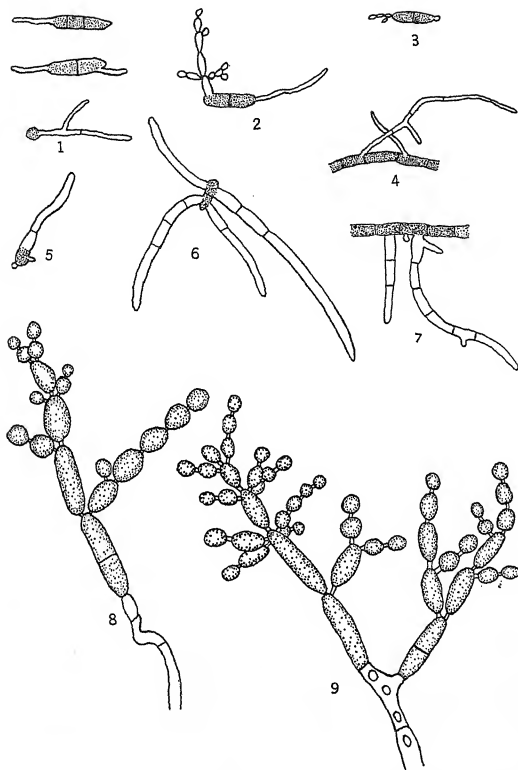
R. C. SPANGLER

(WITH NINE FIGURES)

Cladosporium fulvum Cke. has been observed in the United States since 1883. Although many workers have described it, MAKEMSON (4) was the first to publish an account of its life cycle. During the spring of 1915 this fungus caused much damage to the tomato plants in the greenhouses of the West Virginia Experiment Station. An investigation of the fungus was assigned to the writer by Dr. JOHN L. SHELDON, under whose directions the work was conducted.

When pure cultures of the fungus were examined, many small, almost spherical spores were found, besides the two-celled ones typical of *Cladosporium fulvum*. Both kinds of spores were placed in a hanging drop of sterile water, and in a few hours were germinating. The germ tubes, which were not more than one-fourth the diameter of the large spores, grew rapidly, branched, and became septate (fig. 1). Sometimes short chains of spores developed immediately from the old ones (figs. 2, 3). Probably this occurred only when the germinating spores were near the surface of the water, and the germ tubes started immediately into the air. Cells of the old mycelium also germinated in the same manner as the spores (fig. 4).

A hanging drop of culture medium was prepared and inoculated. Such cultures were kept in good condition for several months. At the end of four hours the spores were beginning to germinate. These germ tubes were about the same diameter as the spores and the mycelial hyphae (figs. 5-7). A mass of much branched, septate mycelium soon developed from each spore. In three days from the time the culture slides were prepared there were many branching conidiophores with chains of fully developed spores. One-celled spherical, and one-celled, two-celled, and occasionally three-celled elliptical spores were produced on the same conidiophore (figs. 8, 9).



FIGS. 1-9.—Fig. 1, germination of spores in water; fig. 2, formation of conidio-spore directly from germinating spore; fig. 3, formation of spores directly from germinating spore; fig. 4, germination of cells of old mycelium in water; figs. 5-7, germination of spores and cells of old mycelium in culture medium; figs. 8, 9, conidiophores and spores (drawn from culture slide); $\times 1700$.

Sometimes as many as six chains of the small oval to spherical spores developed from a large elliptical spore. Small isthmi connected the spores, which apparently developed on all the hyphae as soon as they reached the air.

Sometimes a germinating spore produced a short hypha on which small spores formed directly (fig. 2). The small spherical spores produced mycelium and conidiophores with all the different forms of conidia, just the same as the large two-celled spores and old mycelium. The age of the spores made considerable difference in the time of germination. New spores germinated in four hours, while those eight months old germinated in twenty-four hours. The spores retained their vitality at low and changeable temperatures, as well as at room temperature. They germinated readily after being kept outside in a dry condition from January 4 until May 1. The lowest temperature during this time was -20°C .

Besides growing on the different kinds of culture media, the fungus grew just as well on corn meal that had been moistened and sterilized. Some spores were put on leaves of corn in the greenhouse, and the fungus developed on the leaves that were partially dead. When these leaves were placed in a moist chamber the growth was much more extensive. It also grew and produced spores on tomato leaves removed from the plant and placed in a moist chamber.

These data indicate that *Cladosporium fulvum* is a saprophyte which grows on living plants only when they are placed under abnormal conditions. MAKEMSON says that *C. fulvum* has "ability to exist as a saprophyte. Its growth on culture media is easily obtained even on sterilized filter paper where it grows and produces spores." DUGGAR (2) says that "under abnormal conditions, such as forcing and poor ventilation, many fungi are able to gain entrance and become the cause of epidemics, whereas, under more normal conditions, they remain as harmless inhabitants of dead material." *C. cucumerinum* is supposed to be parasitic only occasionally; *C. herbarum* was thought formerly to be wholly saprophytic; most of the other members of this genus are saprophytes (2).

It has been thought for several years that *Cladosporium* and *Hormodendron* are intimately connected (5). In 1910 BANCROFT (1)

stated that the life cycle of a fungus that he had been studying was composed of the two conidial forms, *Cladosporium* and *Hormodendron*, and that the former was a late saprophytic form on dead leaves that had previously been attacked by the latter. MASSEE (5) says that *Cladosporium epiphyllum* and *Hormodendron Hordei* are different conidial stages of the same fungus. STEVENS (6) gives *H. herbarum* and *H. cladosporioides* as two conidial stages of *Mycosphaerella tulasnei* JANCZ (3).

From what has been discovered in this investigation, it seems certain that *Cladosporium* and *Hormodendron* are one and the same fungus. The one-celled spores near the tip characterize the fungus as *Hormodendron*, while the two-celled spores, after the unicellular ones have fallen off, indicate that this organism is a species of *Cladosporium*. It is probable that these fungi do not possess two types of conidia, but that the two-celled bodies confused as spores are fragments of modified conidiophores. This theory is strengthened by the fact that even unicellular and bicellular pieces of hyphae germinated and reproduced just as typically as the so-called two-celled spores.

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BRIEFER ARTICLES

THE PROPHYLLUM OF GRASSES

(WITH ONE FIGURE)

In a recent article Mrs. ARBER¹ discusses the nature of the prophyllum of grasses, and confirms the position taken by BUGNON that this organ represents a single leaf. The evidence adduced in support of this view may be summarized as follows: (1) the two prophyllary fibrovascular bundles that coincide more or less closely with the two keels are of unequal size; (2) the large bundle occupies a position opposite the median bundle of the next succeeding leaf; (3) as a result of mechanical pressure the prophyllary bundles are not always located in the keels.

According to BUGNON, the smaller prophyllary bundle is a lateral of the first. None of these arguments appears convincing to the writer. If the two bundles in the prophyllum were exactly equal it might indicate a double organ, but inequality can scarcely be said to be incompatible with the idea that two leaves are represented. Is it improbable that two congenitally fused organs, although of the same origin, should have an unequal development? Likewise, the position of the larger bundle opposite the median bundle of the next leaf would seem of little weight, unless it can be shown definitely that the smaller bundle occupies a higher position on the axis.

That the keels in a large measure are the result of mechanical pressure is evident, and that a displacement of the bundle should result seems not improbable. It is not clear, however, how this displacement indicates that both bundles belong to a single leaf.

Opposed to such arguments is the very direct evidence of dual origin afforded by the not infrequent formation of two buds in the axil of the prophyllum of maize.

A type of maize from the Mexican plateau normally produces branches in the axils of the prophylla of the ear stalks, and in certain strains as high as 5 per cent of the individuals afford instances of two buds from the same prophyllum. These prophyllary buds are not mere rudiments, but develop into secondary ears of approximately equal size.

¹ ARBER, AGNES, Leaves of the Gramineae. BOT. GAZ. 76:374-388. 1923.

Prophyllary branches also occur regularly in teosinte (*Euchlaena mexicana*), and cases of two prophyllary branches have been observed in the second generation of a hybrid between maize and teosinte (fig. 1).

When a single bud develops in the axil of a prophyllum it always stands adjacent to one of the keels. No doubt mechanical pressure might displace a bud from its normal position, but mechanical pressure

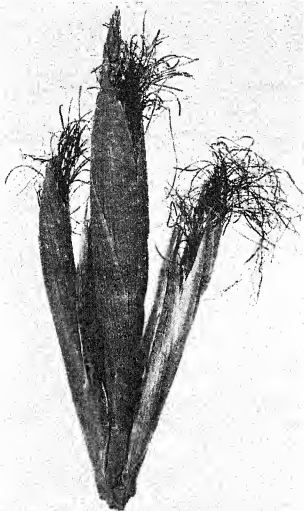


FIG. 1.—Dorsal view of branch of *Euchlaena* × *Zea* hybrid, showing two secondary branches in axil of prophyllum.

would scarcely explain the formation of a second bud. Fusion of adjacent leaves is not infrequent, and there are many examples in aberrant types of maize. In fact, fusion of the leaves usually accompanies extreme shortening of internodes.

It seems more reasonable to suppose that the prophyllum represents two leaves than that two buds should develop in the axil of a single leaf.—G. N. COLLINS, *Bureau of Plant Industry, Washington, D.C.*

CURRENT LITERATURE

BOOK REVIEWS

Electrophysiology of plants

The field of electrical response in plants has been summarized by STERN,¹ as the fourth volume of the series of monographs from the field of physiology of plants and animals published by SPRINGER. The book contains nine chapters, the first of which considers the physical basis of electrophysiology of plants, and the ways in which various forms of energy may be converted into electrical energy in the cell, and how in turn the electrical energy may be converted into mechanical, osmotic, and chemical energy.

The second chapter deals with the effects of electric currents, physical and vital, upon the protoplasm and cell. Electrophoresis, electric osmose, and concentration changes due to electrical phenomena are explained in some detail. Then follow chapters on the quantitative relations between stimulation and response, electrotaxis, electrotropism, and electronasty. These subjects possibly receive more attention than their relative importance would justify, but research has been more general in the field of responses of directly visible kind.

The seventh chapter discusses the influence of electricity on developmental processes and metabolism of plants, and here one wishes that much more information were available. The final chapters deal with the production of electrical energy by the plant, and the problems and tasks of electrophysiological research of the future.

About 200 literature citations are given at the close, arranged in accordance with the various chapter headings. This little volume is interestingly written, and will prove helpful to anyone who desires to obtain a general view of this special field of plant physiology, as well as to the more serious student who may care to investigate the problems of immediate response of living cells to electrical conditions, or the more important developmental and metabolic consequences of the electrical conditions surrounding or pervading the living cells of the organism.—C. A. SHULL.

MINOR NOTICES

Flora Balearica

KNOCHE² has completed his discussion of the flora of the Balearic Isles by adding to the volumes already noted³ one containing a discussion of the floristic and ecological problems, and another of plates illustrating about 50 species

¹ STERN, KURT, *Electrophysiologie der Pflanzen*. 8vo., pp. viii+219. figs. 32. Berlin: Julius Springer. 1924.

² KNOCHE, HERMAN, *Flora Balearica*. *Étude phytogéographique sur les Isles Baléares*. Montpellier. 8vo. 3 and 4: xv+411. pls. 1-47. 1923.

³ BOT. GAZ. 75: 324-325. 1923.

and varieties from the flora, and showing characteristic views of the vegetation of the islands. In sketching the geologic history of this portion of the Mediterranean region, there is postulated a continent in the Eocene stretching from New Zealand through America to the Balearics. The lost continent of Atlantis is also regarded as an established fact, and is called upon to further explain certain affinities. Many of the typical sclerophylls of the Mediterranean, such as *Myrtus*, *Pistacia*, *Rhamnus*, *Buxus*, and *Laurus* are referred to an Eocene origin, with affinities to forms in New Zealand, Chili, Mexico, and California, as well as with the remainder of the Mediterranean floras. The vascular endemics number 61 species, and such groups as relic, recent, Tyrrhenian, and Mediterranean are recognized.

The principal plant association type is an open scrub or "garigue," in which the dominant species are *Pistacia lentiscus*, *Cistus alba*, *C. monspeliensis*, *Rosmarinus officinalis*, and *Olea silvestris*.—GEO. D. FULLER.

Forestry almanac

A small volume⁴ compiled and issued by the American Tree Association, of which CHARLES LATHROP PACK is president, contains information of interest to botanists regarding the forests of our land. Among the subjects included are the history and organization of the United States Forest Service, details regarding various forestry associations now existing throughout the country, and a list of universities and colleges offering educational courses in forestry. The forest taxation laws of various states are summarized, and forest statistics for the different states are presented. In addition there are data regarding the forests of Alaska, Hawaii, Guam, Samoa, and Canada, making it a convenient and indispensable source of information for all who are interested in the present condition of forests in North America and in our possessions beyond the seas.—GEO. D. FULLER.

NOTES FOR STUDENTS

Vegetation of middle west.—Recent publications have tended to clarify our understanding of the grassland region that occupies the center of our continent. GLEASON⁵ holds that these grasslands probably date back to the changes of climate that resulted from the Cretaceous uplift of the Rocky Mountain system, when increasing aridity caused the retreat of forest vegetation. During the Tertiary this grassland flora was probably further differentiated from the elements centering in the Appalachian Mountains, in the Mississippi Embayment, in the Ozark Mountains, in the plains of Kansas and Nebraska, and in eastern Canada. During the various glacial and interglacial

⁴ American Tree Association. Forestry Almanac. viii+225. Philadelphia: J. B. Lippincott Co. \$2.00.

⁵ GLEASON, H. A., The vegetational history of the middle west. Ann. Assn. Amer. Geog. 12:39-85. 1923.

periods fluctuations in its extent have taken place, but it is believed that the occupancy of the Ohio Valley by deciduous forests has been continuous. During the Sangamon interglacial period there seems to have been a considerable western extension of deciduous forests, while during the Wisconsin glaciation a semi-arid climate dominated the region, and was succeeded by a xerothermic period following the retreat of the ice, and causing the invasions of western Ohio by prairies. Increased precipitation then caused the westward migration of forests to be met and combated by the arrival of the Indian and the accompanying prairie fires. During the nineteenth century afforestation again seems to have continued until checked by cultivation.

In the analysis of existing conditions, KINCER⁶ has carefully discussed the moisture and temperature conditions in relation to both natural vegetation and crop production. In a closely related discussion, MARBUT,⁷ postulating the general uniformity of mature soils, whatever the nature of the parent material, throughout any given climate and vegetational area, shows a remarkable agreement between the rainfall regions and the belts of black, dark brown, and brown soils that run from north to south. He has also shown a zone of alkaline salt accumulation, usually lime carbonate, to be characteristic of the mature soils of the middle west, the carbonate layer approaching the surface in the more arid west, but retreating downward and finally disappearing in the humid east.

As a fitting climax to this series of articles, SHANTZ⁸ has described the vegetation of the plains region, relating it to both soil and climate. The chief types of grassland distinguished, described, and mapped are short grass, tall grass, mesquit and desert grass, savanna, sage brush, and mesquite grass. Subdivisions of these types are recognized, and the resulting map is a very decided contribution to our knowledge of the vegetation of the middle west.

The interrelations of vegetation, soil, and climate have an emphasis seldom given. Thus the habitat may be evaluated either by the interpretation of the vegetation or by the interpretation of the soil profile. With no vegetation there would have been no carbonate layer, while the carbonate layer in its turn materially affects the character of the vegetation. Sand equalizes habitats by affording the best conservation of moisture in arid regions and the best drainage in humid climates.—GEO. D. FULLER.

Taxonomic notes.—BALL⁹ has issued a separate of his presentation of the Salicaceae of the Pacific States. Each species is illustrated, helping materially

⁶ KINCER, J. B., The climate of the great plains as a factor in their utilization. Ann. Assn. Amer. Geog. 13:67-80. 1923.

⁷ MARBUT, C. F., Soils of the great plains. Ann. Assn. Amer. Geog. 13:41-66. 1923.

⁸ SHANTZ, H. L., The natural vegetation of the great plains region. Ann. Assn. Amer. Geog. 13:81-107. 1923.

⁹ BALL, C. R., The willows of the Pacific States. Reprinted from *Illustrated Flora of the Pacific States*. pp. 486-507. 1923.

in determination in this difficult group. *Populus* is represented by 4 species, and *Salix* by 46, 6 of which are credited to the author. BALL¹⁰ has also published a new variety of *Salix discolor* from South Dakota, and announced extension of range of five other species.

BLAKE¹¹ has published the results of his study of the Asteraceae as represented in the National Herbarium, supplemented by material in the Gray Herbarium, the herbarium of the Field Museum, and the herbarium of the New York Botanical Garden. The work includes Mexico and South America, especially the northern and western portions of the latter. The nine tribes presented include 118 new species, distributed among 45 genera, the largest numbers occurring in *Perymenium* (19), *Clibadium* (9), *Zexmema* (9), *Verbesina* (9), and *Hieracium* (8). One new genus (*Trichocoryne*) of the Helenieae is established.

HITCHCOCK¹² has published a monograph of the North American species of *Aristida*, recognizing 60 species, 7 of which are described as new. The descriptions are very complete, and include detailed lists of all collections of material. The new species are chiefly from the West Indies, with one from Florida, and one from Lower California.

RYDBERG,¹³ in continuation of his studies of North American Fabaceae, has presented 17 genera of Galegeae, including a new genus (*Sauvallella*) based on *Corynella immarginata* Wright.

TIFFANY¹⁴ has described five new species of *Oedogonium* and one new species of *Spirogyra*, based on collections from Iowa and Ohio.—J. M. C.

Nitrogen fixation by green algae.—Several years ago WANN¹⁵ reported the fixation of free nitrogen by seven species of algae belonging to the Chlorophyceae. The claim that wheat plants have power to fix free nitrogen was made at about the same time by LIPMAN and TAYLOR.¹⁶ The problem of fixation of nitrogen by green algae has been reexamined critically by BRISTOL and PAGE,¹⁷ who have used the same nutritive conditions as WANN employed, but more accurate methods of analysis for nitrogen in the presence of nitrates, namely,

¹⁰ BALL, C. R., Extension of range and a new variety in *Salix*. *Rhodora* 26:135-144. 1924.

¹¹ BLAKE, S. F., New American Asteraceae. *Contrib. U.S. Nat. Herb.* 22:587-661. *pls.* 54-63. 1924.

¹² HITCHCOCK, A. S., The North American species of *Aristida*. *Contrib. U.S. Nat. Herb.* 22:517-586. 1924.

¹³ RYDBERG, P. A., Genera of North American Fabaceae II. Tribe Galegeae. *Amer. Jour. Bot.* 11:470-482. *pls.* 33-36. 1924.

¹⁴ TIFFANY, L. H., Some new forms of *Spirogyra* and *Oedogonium*. *Ohio Jour. Sci.* 34:180-187. *pls.* 3. 1924.

¹⁵ BOT. GAZ. 73:247-248. 1922.

¹⁶ LIPMAN, G. B., and TAYLOR, J. K., Proof of the power of the wheat plant to fix atmospheric nitrogen. *Science N.S.* 56:605-606. 1922.

¹⁷ BRISTOL, B. MURIEL, and PAGE, H. J., A critical inquiry into the alleged fixation of nitrogen by green algae. *Ann. Applied Biol.* 10:378-408. 1923.

the Ulsch in combination with the Kjeldahl method, to avoid the destruction of nitric acid while boiling in the presence of sulphuric acid. This source of error in WANN's work is shown to represent 5-14 per cent of the nitrate present in the culture medium.

Four genera of algae are represented in BRISTOL and PAGE's work, *Chlorella*, *Pleurococcus*, *Cystococcus*, and *Scenedesmus*. The recovery of nitrogen from the cultures by their methods of analytical procedure was 99.28 per cent. and from the controls 99.25 per cent. The authors have failed entirely to corroborate WANN's results. Their concluding sentence puts the case succinctly. "While it is quite conceivable that green algae might assimilate atmospheric nitrogen under certain conditions as yet unknown, there is so far no trustworthy evidence that they can do so."

In regard to the higher non-legumes, like wheat and other Gramineae, the burden of proof that they can assimilate free atmospheric nitrogen rests with those making the claim. The mere assertion that they do cannot be taken seriously until the statement is supported by convincing evidence obtained by methods that eliminate all sources of error. Such data at present are not available.—C. A. SHULL.

Insect pollination in *Aristolochia*.—This genus readily attracts attention because of the unusual shape of the flowers, and often by their bizarre coloring, large size, and unpleasant odor. In general the flowers consist of a basal bulb and an open flaring face connected by a long tube. In most species the face is strikingly colored and ornamented with radial lines converging at the mouth of the tube. This tube is lined with hinged hairs, while the bulb into which it expands possesses transparent spots or "windows" near its base. All these structures seem connected with a rather complicated system of insect-pollination. In order to understand this process more fully, PETCH¹⁸ for several years has been examining these plants in the gardens at Peradeniya.

Of the twelve species investigated, self-pollination obtained in a few, but most were protogynous and dependent upon the visits of various flies for the transportation of pollen from flower to flower. In general the flowers open in the morning, and the insects attracted by the conspicuous face and the vile odor enter the tube, from which their escape is prevented by a lining of hinged hairs. Proceeding to the bulb, the light penetrating the thin tissues of the "windows" attracts them to the vicinity of the stigma and the stamens. The stigmas are receptive as soon as the flower opens, while the pollen is shed late in the day or during the ensuing night. In some flowers food for the insects is found within the bulb, but in nearly all the insects are not liberated until the morning of the second day, when the guardian hairs wilt, leaving a clear passage for the flies to traverse, after which many find their way to a new set of flowers. In promoting the migration the attracting odor usually persists for the first day only.

¹⁸ PETCH, T., Notes on *Aristolochia*. Ann. Roy. Bot. Gard. Peradeniya 8: 1-108. 1924.

In addition to establishing the general behavior just summarized, the report contains an abundance of interesting details of the behavior of different species, and is illustrated by several good plates presenting the details of flower structure. There are also notes on the identity of the insect visitors.—GEO. D. FULLER.

Soil temperature in deserts.—The temperature of the surface soil in deserts is known to vary considerably, although exact data on the subject have been few. SINCLAIR¹⁹ found near Tucson, Arizona, a daily range from 17.0° to 71.5° C. at a depth of 0.4 cm. During the same day the range at a depth of 10 cm. was but 26.3° to 40.1° C., and at 100 cm. there was a constant temperature of 24.5° C. throughout the day. At 4 cm. above surface the range was from 10° C. to 55.7° C.

A somewhat similar range was found by WILLIAMS²⁰ in a valley 12 miles southeast of Cairo, where during August, 1922, the surface of the sand had a daily temperature range of 37° C., with a weekly range of 17.5° to 58.2° C., while in the shade the variations were 21.2° to 35.0° C., and 12 m. within a cave from 24.0° to 25.4° C. only. The daily range of relative humidity was as high as from 14 to 80 per cent.

In Palestine BUXTON²¹ found maximum surface temperatures ranging from 50° to 74° C. He discusses the technique of such surface measurements and the cause of very considerable variations within a very limited space.

From even these scanty data it is evident that desert vegetation has to contend both with very high surface temperatures, and with a wide temperature range for different parts of the same plant, and for different hours of the same day. While this has been known for many years, the investigations cited give some definite idea of the actual temperatures involved.—GEO. D. FULLER.

Alpine vegetation of Colorado.—HOLM²² has recently discussed some 170 species of vascular plants collected in the alpine region of Colorado, and noted the occurrence of many of them in other lands. It was found that 63 species occur also in the Arctic regions, 31 are circumpolar, while of the remainder nearly 100 are endemic to North America, and many to these mountains. Four distinct elements of this vegetation are recognized: the circumpolar, the Arctic, the alpine, and the lowland. Examining the geographic distribution of these alpine plants in detail, the author finds many points that seem to support SCHOUW's hypothesis that the same species has developed at several stations far removed from one another.

The plates are filled with over seventy figures of natural size drawings of some of the more typical species.—GEO. D. FULLER.

¹⁹ SINCLAIR, J. G., Temperatures of the soil and air in a desert. *Mo. Weath. Rev.* 50:142-144. 1922.

²⁰ WILLIAMS, C. B., A short bioclimatic study in the Egyptian desert. *Egypt Dept. Agric. Tech. and Sci. Service Bull.* 19. pp. 20. 1923.

²¹ BUXTON, P. A., The temperature of the surface of deserts. *Jour. Ecol.* 12:127-134. 1924.

²² HOLM, THEODORE, The vegetation of the alpine region of the Rocky Mountains in Colorado. *Mem. Nat. Acad. Sci.* 19. pp. 45. 1923.

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December 1924.

PRELIMINARY STUDY OF RÔLE OF SALTS IN POLLEN TUBE GROWTH¹

R. A. BRINK

The primary purpose of this paper is to present the results of some preliminary investigations concerning the relation of electrolytes to pollen tube growth. Those who have attempted to cultivate pollen *in vitro*, and have sought to improve their culture media by the addition of salts commonly employed in nutrient solutions, will have been impressed with the highly toxic nature of these substances. No case has been reported, so far as the writer is aware, in which it was definitely shown that the addition of salts, either singly or in mixtures, promotes pollen tube growth. While high germination and strong initial growth have been obtained with certain forms in culture solutions containing sucrose only, for example, it is not to be inferred that salts are unimportant in this connection. Indeed, the present evidence points to their being of considerable significance. Before proceeding to a discussion of the specific effects of electrolytes, however, the status of certain other problems in pollen physiology, to which the present investigations are more or less intimately related, will be indicated briefly.

The male gametophyte of angiosperms is dependent during growth upon the pistillate tissue it traverses, in so far as the materials stored in the microspore are insufficient for its complete

¹Papers from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin, no. 45. Published with the approval of the Director of the Station.

development. It would be interesting to know to what extent the materials contributed by the spore must be supplemented. Are the contents of the mature pollen grain so completely organized that the developing gametophyte requires only carbohydrates, these to serve as building materials for the tube structure and as a source of energy? Or are nitrogenous substances demanded as well? What rôle do salts play in maintaining a favorable relation for growth? Studies on artificial media should provide answers to these questions.

Numerous investigations have shown that the pollen of many plants may be germinated in artificial media of relatively simple composition. Indeed, the pollen of some forms requires only suitable moisture conditions for the production of tubes, as VON MOHL (15) first observed, and as JOST (9), MARTIN (13), and ANTHONY and HARLAN (2) have amply confirmed. The pollen of a great many other species may be germinated readily in sugar solutions, especially sucrose solutions. That the rôle of the sugars is not purely a physical one is shown by the fact that certain kinds of pollen that germinate in a cane sugar medium of a particular concentration will not do so in isotonic solutions of glucose or fructose. The writer has found, for instance, that sweet pea pollen gives a high percentage of germination in 0.418M sucrose, but none whatever in glucose or fructose, or mixtures of these in the same molar concentration. This indicates that the production of tubes may be dependent upon a definite chemical stimulus. It is probable that sucrose plays a wide rôle in this connection. MOLISCH (14) observed that the pollen of *Azalea indica*, *Rhododendron ponticum*, and *R. arboreum*, which otherwise would germinate only on the stigma, grew well in weak solutions of malic acid or calcium malate.

The two most important conditions for germination seem to be the maintenance of certain water relations between the pollen grain and its substratum, and the presence, in many cases at least, of a definite chemical stimulus. The chemical stimuli are probably limited to a few widely distributed substances, among which cane sugar appears to be of common occurrence. It has been found by various investigators that pollen of many species germinates on foreign stigmas. This shows that the requirements for germination of pollen from widely different species may not vary greatly.

It is a much simpler matter to induce pollen to germinate on artificial media than it is to maintain the growth of the tubes. In consequence our knowledge of the requirements for complete development is rather meager. STRASBURGER'S (18) observation that the pollen tubes of certain plants would grow readily in the pistils of some very different forms suggests that here too specificity may not be great. The data are far too few at present, however, to establish any conclusion on this point.

The rapidity with which the starch or fatty reserve materials contributed by the pollen grain are digested in the growing tube on artificial media shows that the carbon requirement of the male gametophyte is comparatively high. This becomes intelligible when the relative extent of the wall of the tube secreted is considered. The carbon supply may be the most important element in the nutrition of the microgametophyte.

Little is known regarding the nitrogen requirements of pollen tubes. Possibly, where the gametes are formed in the pollen grain before the latter is shed by the parent plant, and this occurs in certain species among both dicotyledons and monocotyledons (5), the gametophyte may be independent of additional supplies of nitrogen. The mass of protoplasm at no time becomes large, but advances with the tip of the elongating tube, the older portions of the latter being frequently cut off by definite cross-walls, the *Propfen* of STRASBURGER. It is generally believed that of the principal cell parts, the nucleus is the richest in proteins. If the male nuclei are formed at an early period in the history of the gametophyte, the demand for further nitrogen from the conducting tissue may not be important. This may not be true, of course, when the generative nucleus divides in the tube.

Such analyses as are available indicate that the nitrogen content of pollen is relatively high. CZAPEK (6) may be consulted for a summary of the earlier investigations. HEYL (8) found 18 per cent non-protein nitrogenous substance and 30 per cent albuminous material in rye pollen. The same investigator has shown that ragweed pollen, with its three nuclei, contains 24.4 per cent protein. ANDERSON and KULP (1) report that air dried maize pollen contains 4.3 per cent nitrogen. If we employ the factor 6.25 (a rather

arbitrary value in the present case), this is equivalent to 26.87 per cent protein. A definite conclusion regarding the adequacy of the nitrogen stored within the pollen grain for complete development of the gametophyte must await the results of cultural studies on nitrogen-free artificial media.

A systematic investigation of the rôle of salts in pollen tube growth, in keeping with the advances in our knowledge of the general physiological importance of these substances, has never been made. Some results have been published, however, which indicate that their addition to artificial media may be very injurious. LINDFORSS (10) found that NaCl , KNO_3 , and $\text{Ca}(\text{NO}_3)_2$, even in very small amounts, are poisonous to pollen, although different species vary in the amount of injury shown. In a later paper he (11) reports that egg albumin is toxic, but after dialysis will exert a chemotropic effect upon certain kinds of pollen tubes. He attributes the injurious effects of the undialyzed substance to the salts present. TOKUGAWA (19) tested the influence of KNO_3 , K_3PO_4 , CaSO_4 , FeSO_4 , ZnSO_4 , and CuSO_4 , and found all of them toxic to pollen in varying degrees. PATON (17) reports that good growth of pollen from several species was obtained on a medium containing 0.05 per cent MgSO_4 , 0.1 per cent K_2HPO_4 , 0.05 per cent KCl , 0.01 per cent FeSO_4 , and 0.2 per cent agar. No data are presented, however, which show the amount of growth obtained.

While previous investigators have shown that pollen is very sensitive to various neutral salts, but little information has been gained regarding their relative toxicities or the minimum concentrations in which injury is effected. No information has heretofore been available by which the importance of such a variable in artificial media could be determined, nor has any method been suggested by which the toxicity might be overcome. Moreover, nothing has been learned with respect to the rôle of salts in the preservation of the normal permeability of the pollen tube, a matter of paramount importance, perhaps, in cultural methods.

The present paper constitutes little more than a preliminary survey of the subject. Those who have worked in this field are aware of the difficulties involved in getting evidence for an interpretation of the phenomena in quantitative terms. The extraor-

dinary variability in the responses of pollen under seemingly constant conditions in artificial media remains largely unexplained. Attention has been called to this situation in previous papers (3, 4). Under the circumstances large numbers must be used and frequent repetitions made in order to secure significant data. Laborious as this may be, no other way has been found as yet by which the shortcomings of the material in this regard may be circumvented. It seems probable that in several of the experiments here reported significant differences in germination or growth occur as the result of known differential factors in the media; the magnitudes of these differences, however, are subject to large fluctuations due to the vagaries of the material, and the values obtained are to be interpreted with this in mind.

Materials and methods

In the majority of the experiments reported here sweet pea pollen was employed. It is readily procurable in good condition, and usually gives a high percentage of germination under appropriate circumstances. Fifteen per cent c.p. sucrose, in water which had been carefully redistilled into Pyrex glass, formed the basis of the culture media. In view of the probable toxic effect of impurities, the highest grades of chemicals were employed. The pollen was grown in sealed drop cultures at a temperature of 25° C., except in a few of the earlier tests when a temperature control chamber was not available. In each set a check culture containing only sucrose was included. Measurements of pollen tube length were made with an ocular micrometer at a magnification of about 75 diameters. In most of the experiments in which sweet pea pollen was used, the influence of the various salts added was determined by their effect on the amount of growth. Germination counts were made in only a few cases, but in further experiments these values should be regularly obtained also. The *Nicotiana sanderae* pollen employed in the later experiments was so badly shrunken in many cases, and so variable in behavior, that it did not seem pertinent to attempt to secure any more than qualitative data with it. It was hoped, however, that the evidence obtained, taken in conjunction with that from the sweet pea, might serve for the recognition of the more

important issues of the problem, and indicate along what lines quantitative experiments might prove most profitable.

Toxicity of sodium, potassium, and lithium salts

The amounts of growth of sweet pea pollen tubes in cultures containing 15 per cent sucrose and various quantities of NaCl are shown in table I. It will be seen that 0.01M NaCl is so toxic that growth was entirely inhibited at that concentration in two tests, and very greatly reduced in the remainder. At 0.001M the average length of the tubes was less than half that in the salt free check culture. Some toxicity is still in evidence when the amount of NaCl in the media is further reduced fivefold, that is, at 0.0002 M, although the results in Test 2 of the experiment are not in conformity with this.

TABLE I
LENGTH IN MICRONS OF POLLEN TUBES GROWN IN SUCROSE CULTURES
CONTAINING NaCl AS STATED

TEST	CHECK		0.01M NaCl		0.001M NaCl		0.0002M NaCl	
	No.	Length	No.	Length	No.	Length	No.	Length
1.....	160	234	85	39	119	126	137	195
2.....	96	152	35	36	70	87	73	250
3.....	96	284	0	0	60	69	95	155
4.....	120	208	0	0	96	85	96	110
Average.....	472	222	120	38	345	97	401	179

Table II shows the results of a single test with KCl. No germination was obtained at 0.01M, and the average length of the tubes was greatly reduced at 0.002M and 0.001M. At 0.0002M the growth was stronger than in the check culture. In some further experiments in which KCl was employed, no germination was obtained at 0.01M in three tests; at 0.0066M, germination was inhibited in two trials; and a few tubes were produced in a third test. No tubes were produced in two tests at 0.004M, a few in one trial, and high germination was shown in two tests.

The only other salt of a monovalent cation used was LiCl. No germination was obtained at 0.02M in one trial, nor at 0.01M in six trials, nor at 0.0066M in three trials. At 0.004M five tests were

made; no germination was obtained in two cases, a few tubes were produced in one test, and about 50 per cent germination was obtained in each of the other two trials.

TABLE II
LENGTH IN MICRONS OF POLLEN TUBES GROWN IN SUCROSE CULTURES
CONTAINING KCL AS STATED

CHECK		0.01M KCl		0.002M KCl		0.001M KCl		0.0002M KCl		CaCl ₂	
No.	Length	No.	Length	No.	Length	No.	Length	No.	Length	No.	Length
115	234	0	0	57	73	69	86	92	327	111	389

The results concerning the effect of the salts of the monovalent cations Na, K, and Li on growth, taken together with those relating to the concentrations that will just prevent germination, show that the substances in question do not differ greatly in their toxicities.

Toxicity of salts of magnesium, barium, and strontium

In a preliminary test with $MgCl_2$ it was found that germination is entirely prevented at 0.01M, 0.002M, and 0.001M. At 0.0002M, however, growth was obtained equal to that in the check culture. In further experiments designed to determine the concentration of $MgCl_2$ which would just prevent germination, no tubes were produced in two tests at 0.00066M, a very few in one trial, while in four other tests the percentages of germinated grains ranged from 10 to 85.

TABLE III
LENGTH IN MICRONS OF POLLEN TUBES GROWN IN SUCROSE
CULTURES CONTAINING $SrCl_2$ AS STATED

CHECK		0.01M $SrCl_2$		0.002M $SrCl_2$		0.001M $SrCl_2$	
No.	Length	No.	Length	No.	Length	No.	Length
42	381	0	0	69	81	69	496

With $BaCl_2$ no germination was obtained at 0.01M, 0.002M, and 0.001M, with the exception of a few very short tubes produced in one of the six trials made at the last concentration. At 0.00066M no germination was obtained in three trials, about 10 per cent in three

tests, and about 15 per cent in a seventh trial. Less than 5 per cent germination resulted in two tests at $0.0004M$, but in the remaining four trials at this concentration germination was high. At $0.0002M$ little or no toxicity was in evidence. On the basis of these experiments it is concluded that the chlorides of the bivalent cations Mg and Ba are roughly fifteen times as toxic as the corresponding salts of the monovalent cations Na, K, and Li.

That the nature of the cation as well as its valency must be considered, however, is shown by the results with $SrCl_2$, and those with $CaCl_2$ to be presented in the section following. While no germination was obtained with $SrCl_2$ in concentrations of $0.01M$ and $0.002M$, the results at $0.001M$ and $0.00066M$ show it to be appreciably less toxic than $MgCl_2$ and $BaCl_2$. Two tests were made with $0.001M$ solutions; in one no germination was obtained, but in the other most of the grains produced tubes. At $0.00066M$ over 60 per cent germination was obtained in each of two trials. Table III shows the amount of growth of sweet pea pollen tubes in $0.002M$ and $0.001M$ solutions of $SrCl_2$ as compared with the check culture, which contained 15 per cent sucrose only. It will be noted that at $0.001M$ the average length of the tubes exceeds that in the salt free medium. The toxicity at $0.002M$, however, is marked.

Influence of calcium salts

After having tried several single neutral salts which were invariably found to be highly injurious, it was interesting to discover that $CaCl_2$ in corresponding concentrations promoted growth in a very marked degree. The average lengths of sweet pea pollen tubes in cane sugar media containing calcium chloride in amounts ranging from $0.05M$ to $0.002M$ are given in table IV. Increases in growth are shown in $0.02M$, $0.01M$, and $0.002M$. Where $CaCl_2$ is present in a concentration of $0.01M$ this increase amounts to nearly 50 per cent. At $0.05M$ the average for two tests shows somewhat less growth than the check, but the toxicity is not great. A further experiment showed that in the presence of $0.2M$ or $0.1M$ $CaCl_2$ no germination occurs.

In table V the results are given of an experiment to determine the relative growth-promoting powers of three different calcium salts

at two concentrations, 0.01M and 0.002M. It will be observed that the increases in growth in the cultures containing the calcium salts in concentrations of 0.01M are very much greater than those obtained in the preceding experiment. This is due both to a lesser growth in the check cultures and a greater absolute growth in the media containing the calcium salts. Inspection of the separate

TABLE IV

LENGTH IN MICRONS OF POLLEN TUBES GROWN IN SUCROSE CULTURES
CONTAINING CaCl_2 AS STATED

TEST	CHECK		0.05M CaCl_2		0.02M CaCl_2		0.01M CaCl_2		0.002M CaCl_2	
	No.	Length	No.	Length	No.	Length	No.	Length	No.	Length
1.....	90	232	108	257	90	269
2.....	96	331	95	251	95	365	92	625	96	451
3.....	48	144	72	152	72	264	72	243	48	312
Average..	234	254	167	209	167	321	272	378	234	353

TABLE V

LENGTH IN MICRONS OF POLLEN TUBES GROWN IN SUCROSE CULTURES
CONTAINING CaCl_2 , $\text{Ca}(\text{NO}_3)_2$, OR CaSO_4 AS STATED

TEST	CHECK		0.01M CaCl_2		0.002M CaCl_2		0.01M $\text{Ca}(\text{NO}_3)_2$		0.002M $\text{Ca}(\text{NO}_3)_2$		0.01M CaSO_4		0.002M CaSO_4	
	No.	Len.	No.	Len.	No.	Len.	No.	Len.	No.	Len.	No.	Len.	No.	Len.
1.....	103	49	31	689	65	118	35	973	62	106	55	984	56	398
2.....	72	154	38	767	72	208	48	778	65	325	72	446	38	1003
3.....	72	156	44	986	72	418	33	910	61	529	20	1063	24	1215
Average..	247	97	113	831	209	276	116	875	188	319	147	731	118	759

values obtained for each medium in each of the three tests will afford some idea of the variability encountered in such experiments as these. The main facts, however, stand out rather clearly. At 0.01M, CaCl_2 , $\text{Ca}(\text{NO}_3)_2$, and CaSO_4 give average amounts of growth of similar magnitudes. The lengths of the pollen tubes in the 0.002M culture of CaCl_2 and $\text{Ca}(\text{NO}_3)_2$ are likewise in fairly close agreement. With 0.002M CaSO_4 , however, the average length of the tubes is much greater. Since the three tests agree in this regard,

the results are possibly significant, but the point should be tested further.

The data relating to the effect of the chlorides of sodium and potassium as compared with the same calcium salt indicate that in the phenomena under discussion the cation plays the dominant rôle. As a whole the results of the experiments with different calcium salts support this view, although the evidence is not unequivocal, as shown by the 0.002M CaSO_4 cultures.

Protective action of calcium in salt mixtures

Having demonstrated that calcium salts in sucrose cultures showed no toxicity to sweet pea pollen except when present in concentrations greater than 0.04M, and that in more dilute solutions their effect on growth was decidedly salutary, attempts were made to determine whether they exerted any protective effect in the presence of other salts known to be injurious when used singly.

TABLE VI

LENGTH IN MICRONS OF POLLEN TUBES GROWN IN SUCROSE CULTURES
CONTAINING NaCl OR CaCl_2 OR MIXTURES OF THESE SALTS

TEST	CHECK		0.01M NaCl		0.04M CaCl_2		0.01M CaCl_2		0.01M NaCl + 0.04M CaCl_2		0.01M NaCl + 0.01M CaCl_2	
	No.	Len.	No.	Len.	No.	Len.	No.	Len.	No.	Len.	No.	Len.
1.....	105	161	0	0	102	170	96	283	84	303	105	389
2.....	72	118	0	0	72	185	72	659	72	270	72	375
3.....	48	100	0	0	72	229	72	521	72	188	72	452
4.....	72	306	69	98	95	571	94	186	96	408
Average.	297	177	0	0	315	174	335	497	322	236	345	405

The results of experiments with mixtures of NaCl and CaCl_2 are given in table VI. In this series of tests, cultures containing the respective single salts in corresponding concentrations were also included, except in Test 4, where the 0.01M NaCl member was lacking. The results are illuminating. No germination was obtained in any of the cultures containing 0.01M NaCl alone. On the average, the growth in the presence of 0.04M CaCl_2 was nearly equal to that in the salt-free check cultures. In the media containing 0.01M NaCl + 0.04M CaCl_2 the pollen tubes averaged over 30 per cent

longer than the check culture. Not only is the toxic effect of the NaCl prevented, but the growth exceeds that in the 0.04M CaCl₂ culture by about 35 per cent. That is, the toxicity of both salts is reduced when they are combined. The injurious effect of 0.01M NaCl is also overcome when the amount of CaCl₂ is reduced to 0.01M. The average length of the tubes with this combination of salts, however, is nearly 20 per cent less than when 0.01M CaCl₂ alone is present.

TABLE VII

LENGTH IN MICRONS OF POLLEN TUBES IN SUCROSE CULTURES CONTAINING
0.02M KCl AND CaCl₂ AS STATED

TEST	CHECK		0 CaCl ₂		0.001M CaCl ₂		0.002M CaCl ₂		0.01M CaCl ₂		0.02M CaCl ₂		0.04M CaCl ₂	
	No.	Len.	No.	Len.	No.	Len.	No.	Len.	No.	Len.	No.	Len.	No.	Len.
1.....	45	63	0	0	0	0	24	66	60	402	30	278	25	96
2.....	53	86	0	0	45	91	62	99	46	564	49	589	70	87
3.....	70	81	78	50	59	67	93	90	45	614	36	560	65	93
4.....	72	119	0	0	56	47	72	97	35	617	34	736	0	0
5.....	52	74	37	81	38	47	64	117	72	391	48	464	72	132
6.....	70	332	0	0	72	94	72	119	72	255	72	544	72	107
Average...	362	134	115	60	270	71	387	101	330	442	269	535	304	105

In two further experiments attempts were made to determine the amounts of CaCl₂ necessary to render KCl harmless when the latter salt was present in concentrations of 0.02M and 0.04M respectively. Table VII shows that when 0.02M KCl cultures are made 0.01M and 0.02M for CaCl₂, not only is the injurious effect of the former substance overcome, but the growth is three to four times as great as in the salt free check medium.

Three tests were made with the same concentrations of CaCl₂ in cultures containing 0.04M KCl. The results are presented in table VIII. Relatively long pollen tubes were produced in the check culture in Test 2, bringing the average for that medium considerably above the value for the corresponding medium in the previous experiment. It will be noted that when the amount of CaCl₂ present is reduced to 0.001M it is no longer effective in overcoming the toxicity of 0.04M KCl. In the cultures containing 0.04M KCl+0.01M CaCl₂ the average growth exceeds that in the

check medium. When the amount of CaCl_2 is reduced to 0.002M, however, the length of the tubes is only about one-third that in the culture containing sucrose only. In the 0.04M $\text{KCl} + 0.02\text{M CaCl}_2$ cultures, contrary to expectation, the toxicity is rather marked.

TABLE VIII

LENGTH IN MICRONS OF POLLEN TUBES IN SUCROSE CULTURES CONTAINING
0.04M KCl AND CaCl_2 AS STATED

TEST	CHECK		o		0.001M CaCl_2		0.002M CaCl_2		0.01M CaCl_2		0.02M CaCl_2		0.04M CaCl_2	
	No.	Len.	No.	Len.	No.	Len.	No.	Len.	No.	Len.	No.	Len.	No.	Len.
1.....	39	137	o	o	o	o	66	75	72	312	72	135	57	65
2.....	72	536	o	o	o	o	48	65	72	587	72	219	72	85
3.....	36	234	o	o	o	o	42	180	48	587	54	325	72	78
Average...	147	356	o	o	o	o	156	100	192	484	198	218	201	77

In view of the variability in the results of the several tests, additional data will have to be obtained before definite conclusions can be drawn regarding the quantitative aspects of these relations.

Effects of salts on pollen of *Nicotiana sanderae*

A study was made of the effects of salts on the pollen of *Nicotiana sanderae*, in an attempt to determine to what extent the findings in regard to sweet pea pollen might have general validity. The available *Nicotiana* pollen, however, was not well suited to the purpose. Many of the grains were defective and evidently incapable of producing tubes, and possibly an appreciable percentage of those which appeared morphologically complete were likewise non-germinable. Since no other species was at hand at the time, however, the *Nicotiana* pollen was employed. The values given for germination percentages are only approximate, being based upon rough counts under the microscope of two or three fields in each culture.

The results were highly variable, but a sufficient number of tests were made to establish certain facts rather definitely. LiCl , NaCl , and KCl proved injurious to *Nicotiana* pollen in approximately the same degree as had been found for the sweet pea. There were

greater differences, however, between the effects of the three different salts. LiCl was the most toxic, preventing germination entirely at 0.0066M ; NaCl was equally toxic at 0.013M to 0.01M ; while KCl permitted sporadic germination even at 0.02M .

It was interesting to find that the reactions of *Nicotiana* pollen to CaCl_2 and MgCl_2 were quite different from those of the sweet pea. It will be recalled that CaCl_2 exerted a markedly stimulating effect upon the pollen tubes of the latter form. In the case of *Nicotiana*, while 0.01M CaCl_2 gave 50–70 per cent germination in different tests, vigorous tubes were never developed. The toxic effect on the pollen tubes was marked, even in 0.002M CaCl_2 cultures. On the other hand, high germination and strong growth were repeatedly obtained in cultures containing 0.002M MgCl_2 . The percentage of germination was not lowered when the concentration of MgCl_2 was increased to 0.01M , although the average length of the pollen tubes was less. The similarity of the effect of MgCl_2 on *Nicotiana* and CaCl_2 on sweet pea may mean that their action in the two cases is analogous.

BaCl_2 , as in the case of the sweet pea, proved very toxic to *Nicotiana* pollen, inhibiting germination entirely at 0.0005M , and permitting it only in very meager amounts at 0.0004M . SrCl_2 was somewhat less injurious. No germination was obtained at 0.002M in three tests, or at 0.001M in three tests. In three other trials at the latter concentration, however, about 5 per cent germination was obtained in two cases, and 50 per cent in the third. In four cultures containing this salt in a concentration of 0.0005M , the percentages of germination ranged from 10 to 80, but the tubes produced were all very short.

AlCl_3 and $\text{Ce}(\text{NO}_3)_3$, were found to be extremely toxic to *Nicotiana* pollen. Four tests with the former salt and five with the latter showed complete suppression of germination at 0.0004M . At 0.0002M , $\text{Ce}(\text{NO}_3)_3$ gave no germination in three trials and about 50 per cent in one trial. No germination was obtained at 0.0002M in three tests with AlCl_3 ; in three other cases about 10 per cent was observed; and in three further tests gave from 25 to 50 per cent.

Several trials were made also with salts of the heavy metals Cu , Ni , Zn , Mn , and Fe . CuCl_2 , CuSO_4 , and NiCl_2 completely

inhibited germination at $0.0004M$. Very sparse germination was obtained in one-half the trials at $0.0002M$, and none in the remainder. While highly toxic at $0.0005M$, $ZnCl_2$ exerted a markedly stimulating effect at $0.000266M$ and $0.0002M$. At these concentrations growth was usually stronger than in the salt-free check medium. This observation is in accordance with the results others have obtained with various plants grown in dilute solutions of zinc salts. $MnCl_2$ also was found to stimulate growth very appreciably at $0.002M$ and $0.001M$. The pollen tubes produced in the manganese cultures were regularly coiled in a characteristic spiral fashion. At concentrations of $0.0004M$ and $0.0002M$, $FeCl_2$ gave sporadic germination and usually very short tubes.

Discussion and summary

The most striking feature of these results is the extreme sensitivity shown by pollen tubes to salts. It has been demonstrated that $NaCl$, one of the least toxic of the neutral salts tried, excepting $CaCl_2$, added to a sucrose solution in a concentration of $0.0002M$, or about 11 parts per million, reduces the growth of sweet pea pollen tubes 15 per cent. A concentration of $0.01M$ $NaCl$ inhibits germination entirely. $MgCl_2$ and $BaCl_2$ are roughly fifteen times as toxic as $NaCl$. *Nicotiana* pollen is likewise highly susceptible to the injurious effects of single salts, although in certain cases its reaction differs considerably in degree from that of the sweet pea. Possibly this hypersensitivity is due to very rapid penetration of the protoplasts by the substances in question.

It has been established that in the presence of calcium salts in concentrations ranging from $0.02M$ to $0.002M$, the growth of sweet pea pollen tubes is markedly enhanced. $MgCl_2$ has a similar action on *Nicotiana*. So far as the writer has been able to detect by microchemical means, these salts do not affect the wall of the pollen tube; presumably they act on the protoplast itself. In the light of recent experimentation (16) it is not unlikely that the beneficial influence of these salts is related to changes effected in the permeability of the protoplasts. Evidence has been obtained from other forms that the maintenance of normal permeability, an important condition in vital phenomena, is intimately related to the salt content of the

surrounding solution. Possibly in pure sucrose cultures exosmosis is a limiting factor in pollen tube growth; the addition of salts of calcium or magnesium may favor development by retarding or preventing this outward diffusion.

In the protective action exhibited by calcium in solutions containing another salt known to be highly injurious when present alone, we undoubtedly have a well marked case of antagonism. This interpretation is in harmony with the writer's suggestion that the dominant rôle of calcium is in preserving normal permeability, and that it does not act as a true nutrient. The necessity of balancing electrolytes in culture media has been widely recognized, but this phase of pollen physiology is singularly barren of facts. Much improvement might be made in cultures for pollen with a fuller knowledge of these relations.

It is by no means improbable that the pollen tube is independent of external sources of salts, so far as these latter are required as nutritive elements in the narrow sense of the term. The present results would indicate that electrolytes present in the style may play an important rôle, however, in regulating the diffusion of substances through the surface layer of the protoplast of the pollen tube. EAST (7) found that when compatible and incompatible pollen tubes are growing in a single pistil in self-sterile *Nicotiana*, the former group only functions in reproduction. This shows, as EAST has pointed out, that the reaction between pollen tube and surrounding cells must be local. Each sort of microgametophyte maintains its distinctive character in a common environment. Whatever may be the nature of the reaction set up between the compatible tubes and the conducting tissue, enabling the former to complete their development, the adjacent incompatible tubes do not benefit thereby. This difference in the chemical processes of compatible and incompatible tubes is made possible by the existence of semi-permeable membranes. It is not unreasonable to assume that, so far as pollen tube growth is concerned, the chief function of the salts in the style is the preservation of the semi-permeable nature of the protoplasmic membranes.

In connection with these findings on the effects of salts, it is interesting to note certain facts regarding the mode of growth of the

pollen tube in the style. PATON (17) has demonstrated that pollen contains pectinase. It is the secretion of this enzyme, presumably, that enables the pollen tube to pursue its intercellular growth, since the middle lamella, as the work of MANGIN (12) shows, consists of pectose in combination with inorganic bases, principally calcium. In the digestion of the middle lamella these bases would be set free. In view of the pronounced effect of calcium upon the growth of the pollen tube of sweet pea, the digestion of the intercellular substance in the style may have a significance in the development of the male gametophyte not hitherto suspected.

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ANATOMY OF CHEILANTHES TENUIFOLIA

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(WITH PLATES VIII-X)

Introduction

Cheilanthes tenuifolia Swartz grows in all the dry parts of New South Wales, and is a common member of the xerophilous flora. It has adapted itself to situations in which it may be absolutely dry for several months during each year, but as soon as rain comes it commences to grow immediately. The material for the present investigation was collected at Kuring-gai Chase and Picton, New South Wales.

The fern is not very large. It consists of a subterranean dorso-ventral rhizome which occasionally branches and attains a considerable length. Stiff erect fronds rise above the surface of the soil here and there, usually in clumps. This is due to the fact that the fronds grow out from the rhizome much more quickly than the rhizome itself grows. Meanwhile, the fronds along the older parts of the rhizome die, so that there remains a clump of leaves around the apex. There is a very efficient root system growing out from the rhizome, the main roots branching profusely and giving rise to a very extensive secondary root system. The roots are dark brown, and very thin and wiry.

The older portions of the rhizome are covered by the leaf bases of the older leaves, and numerous scales. The young leaf shows circinate vernation, and in the early stages is covered with scales. These, however, fall off as the leaf expands.

The petiole of the leaf is about 6 inches long, thin and wiry, black, and polished. It is quite smooth for the greater part of its length, but toward the base a few scales occur. Midway along the length of the petiole pinnae arise. The frond is tripinnatifid, the pinnae being arranged in numerous alternate pairs. HOOKER and BAKER (7) regarded them as being arranged in numerous opposite pairs, but the writer's observations did not confirm this.

The pinnae are arranged very close to one another and appear to be opposite, but the vascular bundles come off alternately. The rachis is polished like the petiole, and is the same color. Both surfaces of the pinnae are green and naked, and the margins are inrolled to form an indusium. The veins arise from a central midrib, are forked, and bear sori on their ends.

The material was fixed in the field in a chromo-acetic solution. After fixation the material was thoroughly washed, dehydrated, and passed in the usual way into paraffin. Sections were then made of the various parts, the thickness varying from $3\ \mu$ to $10\ \mu$, according to the part of the plant which was being cut. The sections were then stained by one of a number of straining processes, but Fleming's triple stain proved by far the most satisfactory for every part.

Investigation

RHIZOME

The rhizome is very long and thin, covered for the greater part of its length by the leaf bases and scales. It is dorsiventral, with stiff erect fronds and thin wiry roots. BOWER (1) considers that creeping rhizomes are probably derivative. "That large leaved forms would be mechanically unstable is obvious, especially when the stem is thin and the internodes of appreciable length. There is an inherent probability that such axes should become oblique or prone." The rhizome occasionally branches, these branches always bearing the same structure as the main axis. From the condition seen in fig. 1, it would appear that branching is dichotomous, although monopodial branching is of common occurrence among the Polypodiaceae as a whole.

Numerous young leaves arise around the apex. These show circinate vernation, and curl around the apex of the rhizome, thus forming some protection for the latter.

A section of the apex of the stem shows that it becomes hidden by scales, which grow out from the epidermis, thus forming a still more efficient protection for the apex. The termination of the stem is broad and flat, and from this the apex arises rather abruptly as a small cone, terminated by a tetrahedral apical cell (figs. 1, 2). Segments are cut off from two cutting faces, and from these are

formed the older tissues of the rhizome, also the roots and leaves. These segments, cut off parallel to the two cutting faces, are again divided by a wall perpendicular to the long axis of the cell, forming an outer and an inner cell. The former is again divided, so that there are two outer and one inner cell formed from each segment. The inner cell goes to form the vascular cylinder, the outer cells form the cortex and epidermis. The vascular tissue is delimited from the ground tissue to a point very near the apex.

SACHS states that creeping stems with a bilateral structure have a two-sided apical cell, while the erect ascending stems with rosettes and leaves radiating in every direction have a three-sided pyramidal apical cell. KLEIN, however, later disproved this generalization.

More recently BOWER, as a result of his observations in the meristems of a large number of ferns, has stated that in the leptosporangiate ferns, whether radial or erect, the apex is occupied by a three-sided pyramidal apical cell. This generalization, however, is not without its exceptions among dorsiventral forms such as *Pteris aquilina*, *Salvinia*, and *Azolla*, in which a two-sided apical cell is present. *Cheilanthes tenuifolia* is another exception to this rule, for here also a two-sided apical cell is present.

The stem anatomy of the various species of *Cheilanthes* is of interest, in that it contains both solenostelic and dictyostelic types, and forms which are transitional between these two. A section of the rhizome of *C. tenuifolia* shows it to be a dictyostelic type, according to GWYNNE-VAUGHAN'S (5) definition, that is, the continuity of the vascular cylinder is interrupted at more than one point by overlapping leaf gaps (fig. 4). The cortex of the stem becomes transformed into sclerenchyma, and from the outer layers of this scales arise which protect the rhizome. The pith also becomes sclerized, so that sections show a mass of sclerenchyma surrounding a varying number of meristeles.

The detailed anatomy of the stelar tissues furnishes results of considerable interest (fig. 5). The center of the meristeles is occupied by xylem accompanied by wood parenchyma. The xylem is composed wholly of scalariform tracheids; in no instance were there any spiral or annular tracheids corresponding to the protoxylem. Sections were made of the apical region and thoroughly examined, but

even here no trace of protoxylem was observed, the first tracheids to be lignified being large scalariform elements. This condition was also observed by MARSH (9), who noted that there was no protoxylem present in the stems of *Cheilanthes Fendleri*, *C. gracillima*, *Pellaea andromedaefolia*, and other xerophilous forms.

This absence of protoxylem in the vascular bundles of stems is to be found in various other members of the Filicales. BOWER (2) states that in *Lophosoria* "the tracheids are of uniform character, and, as is often the case in the Cyatheaceae, there is no protoxylem. Very soon after leaving the axis, protoxylem groups make their appearance in the vascular strand which had hitherto been without them." GWYNNE-VAUGHAN (6) states:

There are a large number of ferns in which the protoxylems of the petiolar bundles die out toward the base of the trace, disappearing entirely before or immediately after its insertion upon the stele of the stem. No definitely localized protoxylem strands are to be found in the stem of these ferns, nor are there any spiral or annular tracheids present.

He maintains that the first formed scalariform elements of the xylem, which are usually smaller than those formed later on, in these forms, may be considered to represent protoxylem elements. The walls of the scalariform tracheids have a well defined beaded appearance (fig. 7).

It was observed that with the tracheary tissue is mingled more or less wood parenchyma. MARSH concludes that there is a close relation between the stelar structure and the amount and arrangement of wood present within the stele. He observed that in perfectly solenostelic types of *Cheilanthes*, such as *C. Fendleri*, and in types which scarcely depart from this, such as *C. gracillima*, the xylem forms a continuous band of tracheids, not broken by parenchyma. *C. lanuginosa*, which is intermediate between these types and large stemmed dictyosteles, has a wider band of tracheids slightly broken by parenchyma. *C. persica*, a dictyostelic form, has a broader band of wood, in which considerable parenchyma occurs. *C. tenuifolia*, therefore, gives additional support to these statements.

Separating the xylem and phloem are a few layers of parenchyma, the "vasal parenchym" of STRASBURGER. The phloem is

both external and internal, the amount of external being about equal to the amount of internal phloem; nevertheless there is very little phloem present in the stem. The total number of sieve tubes in no instance is large. There is no differentiation into metaphloem and protophloem; the elements are narrow and arranged in a single row on either side of the xylem ring. The phloem ring is not continuous, but is interrupted at the leaf gap (fig. 6). MARSH observed that a similar condition occurred in other species of *Cheilanthes*.

In *C. tenuifolia*, surrounding the whole meristele is a layer of brown cells, differing from the outer sclerenchyma, which constitute the endodermis. Separating the phloem and endodermis are three, four, or perhaps five layers of fairly large cells containing considerable granular matter. The only interpretation of these cells is that they represent the pericycle, which in this instance, therefore, is many layered.

CAMPBELL (3) mentions that in certain types of Filices the pericycle may be double, and usually contains considerable starch. According to STRASBURGER these cells do not constitute a true pericycle, but belong to the cortex. They are the sister cells of the endodermis, which is thus not the innermost cortical layer, but the next but one. This many layered pericycle probably acts as a storage system for the reserve foods of the plant. By this means, as soon as conditions are again suitable for growth, the food materials are available.

The root trace is given off from the outer margin of the stele and passes through the cortex with the minimum of disturbance.

The leaf trace departs from the vascular cylinder as a single C-shaped bundle, and passes through the cortex of the stem in the same condition, associated with sclerenchyma from the pith. The leaf trace is given off from the center of the meristele. This single arched condition of the leaf trace observed in *C. tenuifolia* is of considerable interest, since it indicates that although the stem possesses a dictyostelic structure, it is a primitive member of these types, and dictyostely has just evolved within the group. The type of leaf trace which is present is characteristic of solenostelic types.

GWYNNE-VAUGHAN (6), speaking of the evolution of the dictyostele, states that "an examination of transitional types makes it

clear that the dictyostely of the Cyatheaceae and Polypodiaceae is primarily due simply and solely to the overlapping of the leaf gaps in the solenostele."

Briefly summarizing his conclusions, TANSLEY (11) states as follows:

The leaf trace of the petiolar vascular system of ferns, though correlated in a general way with the structure of the stem stele, has a semi-independent course of origin which it is necessary to consider separately. GWYNNE-VAUGHAN has done good service in calling attention to the fact that the arched type of leaf trace, or some clearly derived form, is to be found in the vast majority of ferns, and is certainly to be regarded as typical. As we pass from protostelic to solenostelic forms, we find the leaf trace taking on a corresponding expansion. BOODLE has attributed the origin of the solenostele, with its increased diameter, to the necessity for accommodating the insertion of broader leaf traces, thus implicitly recognizing the fact, which seems to be indisputable, that the leaf trace leads and the stele follows in the course of evolution.

The results of the present investigation do not appear to confirm the latter part of this statement, since, although the leaf trace is still in the simple condition typical for solenostelic types, the stem has advanced and a perfect dictyostele has been produced. The general rule is that dictyostely is accompanied by the division of the single strand into many.

BOWER (1) states:

All the more primitive types of ferns . . . have a more or less horse-shoe shaped trace, but the modern Marattiaceae, and the bulk of the Polypodiaceae forms have a trace composed of many strands; these are however arranged in a series corresponding to the outline of the undivided trace. These facts indicate, with no possible uncertainty, that there has been disintegration of the leaf trace by fission; it finds its origin in the enlarged upper region of the leaf, and has been phyletically progressive from a region lying towards the base. Thus in several distinct phyla it is shown that a progressive disintegration of the axial stele has followed the expansion of the axis.

It seems clear that the former part of TANSLEY's statement is the more correct, namely, that the leaf stele has a semi-independent course of origin, which it is necessary to consider separately.

Another feature of interest with regard to the leaf trace is that its separation from the stem stele is not effected synchronously on both sides, one side separating before the other. BOWER (2) has observed

that this also occurs in *Lophosoria*, a solenostelic type of the Cyatheaceae, and claims that it is usual in ferns with a creeping axis.

A study of the stem anatomy of *C. tenuifolia* places it among the phyllosiphonic type of JEFFREY'S (8) classification, that is, those types in which the leaves are given off with profound disturbance of the cauline system.

LEAF

The leaf arises from the segments produced by the apical cell of the stem. It first becomes evident as a small conical emergence, at the apex of which is a wedge-shaped apical cell. Segments are cut off parallel to the two cutting faces of this cell. Each segment becomes divided by a transverse wall into an outer and an inner cell. The outer cell becomes further divided by a longitudinal wall. The superficial cells thus produced may give rise to pinnae, growth being due in this instance to a marginal cell. The growth of the leaf is always more rapid on one side than it is on the other, so that the apex tends to become curved inward. This uneven growth gives rise to the circinate vernation of the young leaf, and thus the young axis is protected (fig. 8). BOWER asserts that in the Polypodiaceae the young apical cell is three-sided, but later it becomes two-sided. *C. tenuifolia* is an exception to this statement, for in every instance the apical cell was two-sided even in the young stages.

Petiole

Fig. 9 represents a section typical of the petiole. It will be observed that the petiolar stele forms a single strand, and remains in this condition along its whole length except where branches are given off to the pinnae. This single strand is surrounded by cortex and epidermis, which, as the leaf grows, becomes transformed into sclerenchyma, thus giving the mechanical support necessary, since the petiole itself is thin and wiry. The contour of the petiole coincides mainly with that of the vascular strand. The latter is chiefly composed of xylem, consisting of both protoxylem and metaxylem. When the leaf trace becomes separated from the stem stele it is a simple, adaxially curved, anarch structure, and protoxylem groups make their appearance within it. The protoxylem elements are composed of spiral and annular tracheids. There are

three protoxylem groups occurring in the petiole, two of which are endarch in position, the other in a few instances medianly attached and exarch, but in sections made of the older petiole, medianly placed but detached.

Fig. 10 indicates a condition which seems to be the earliest occurring in the petiole after the simple, adaxially curved, anarch leaf trace, and is found at the base of the petiole of the young leaf. Here are represented three protoxylem groups, two of which are endarch and lateral. The third medianly situated exarch group seems to have divided the metaxylem elements, for the greater part unligified as yet, into two distinct groups separated by parenchyma and the protoxylem group. Fig. 11 represents the condition found in the older stem. There the metaxylem elements have become grouped into a single continuous band, and the third protoxylem group has become detached from the remainder of the xylem elements.

The most feasible explanation of the manner in which these conditions have been attained is to be obtained by a careful study of the types already described by MARSH. He maintains that in all the species which he investigated there is at the base a simple, adaxially curved, anarch leaf trace, but as the petiole is followed upward the changes which occur vary considerably. In the simplest expression three endarch protoxylem groups are developed in this simple bundle; the median group migrates and takes an exarch position, and finally becomes detached altogether. The various stages which MARSH has described as occurring in *C. Fendleri* are not all to be observed in *C. tenuifolia*, but this may be due to secondary simplicity of the petiolar structure, resulting in the dropping out of various types at the base.

MARSH claims that these facts admit of only one explanation, that the base of the leaf preserves primitive characters. This is also the position taken by SINNOTT (10), who has pointed out that the leaf traces of all ferns, with a few exceptions, can be derived from a triarch-mesarch leaf trace, and that the nearest approach to this condition is usually to be found at the leaf base. The mesarch protoxylems in the course of evolution have gradually become endarch, and in many forms all trace of mesarchy is lost,

even at the leaf base. This is the condition found at the leaf base of *C. Fendleri*, and it is only by assuming that the acropetal succession recapitulates the evolutionary succession that we can reasonably bring the peculiar petiole of *C. Fendleri* into line with the normal filicinean leaf trace.

The actual cause of the exarchy and detachment of the median protoxylem group is so far unexplained. It has been claimed by many investigators that it is at the base of the petiole that any change will occur first, since it is here (as the leaf in the evolutionary ascent enlarges) that the greatest demand for an increased water supply will be most keenly felt. Fig. 10 seems to indicate that the single strand of the petiole is becoming double, but the separation into two bundles has not actually taken place. A break in the protoxylem group, which forms the sole link between the two distinct xylem masses, would mean that two distinct xylem strands would exist, each with two protoxylem groups, and in many instances this was seen to have occurred. The phloem ring surrounding the xylem in a few instances is broken, but the endodermis and pericycle are still continuous. It would seem that this serves to indicate how a doubling of the petiolar bundle may have occurred. The condition found higher in the petiole is quite in harmony with this; the demand for water is not felt so keenly here as lower down. The metaxylem elements have become continuous, and the median protoxylem group has become detached.

The condition in which there exists a double trace at the base, which unites to form a single strand at a higher level, is to be observed in other genera, such as *Onoclea sensibilis* and *Diacalpe*.

The metaxylem is composed of scalariform tracheids of the same nature as those occurring in the stem. Here also there are a few layers of parenchyma separating the xylem and phloem. The phloem is poorly developed, consisting of a single row of sieve tubes surrounding the xylem, but absent from the sides of the bundle and also from the vicinity of the median protoxylem group (figs. 12, 13). The same explanation may be applied to the poor development of the phloem in the petiole, as was mentioned in the case of the rhizome. The vascular strand may be regarded as concentric, the absence of phloem on the sides being due to reduction.

Surrounding the phloem are two or three layers of parenchymatous cells containing dense granular cytoplasm. These, as in the rhizome, might be interpreted as representing a many layered pericycle, the cells storing quantities of reserve food material. This is surrounded by the endodermis of the same nature as that of the rhizome. The cortex is sclerenchymatous.

When the petiole reaches the level of the pinnae, the vascular strand gives off branches which enter the rachis, each rachis containing a single strand of the same nature as that of the petiole. This again branches in the rachis and supplies the vascular strands to the pinnules.

Pinnules

The veins of the pinnules always show dichotomous branching, and on the ends of these forked veins sori are borne. No hairs or scales are present, although they occur in the bud. The minute anatomy of the pinnule is represented in fig. 14. The upper epidermis is composed of large cells, protected by a cuticle. The stomata are confined to the lower epidermis, and are protected by the inrolled margins of the leaf alone. In this respect *C. tenuifolia* is similar to the xerophilous fern *Pellaea andromedaefolia*, described by MARSH. Other xerophilous species of *Cheilanthes* have the stomata protected by the inrolled margins and a covering of scales, or by hairs. A surface section of the lower epidermis (fig. 18) indicates the nature of the stomata. The guard cells contain chlorophyll, granular cytoplasm, and a large nucleus. The cells of the lower epidermis have a convoluted outline.

The palisade tissue is well developed, and is two or three cell layers deep. The cells have the chromatophores distributed around the margins of their walls. The lacunar tissue is reduced, the cells of which may contain chromatophores.

The vascular system of the lamina is supplied by veins, which run out obliquely from the midrib of the pinna. The vascular bundles have a well marked endodermis (fig. 16), the cells of which are large, colorless, and thin walled. Below this is a single (sometimes a double) layer of cells of identically the same nature as those already described as occurring beneath the endodermis of the stem and petiole. These cells may be regarded as part of the pericycle

in which reserve food is stored until it is required by the plant. A single layer of sieve tubes representing the phloem surrounds the xylem, although at intervals its continuity is interrupted by parenchymatous cells. The vascular supply to the developing sporangia is represented by a vascular commissure which runs below the whole area of the sorus.

These features indicate that in the leaf there are well marked adaptations both in texture and in the minute anatomy to the habitat of the fern.

Root

The root system always arises in connection with the bases of the leaves, and consists of a number of delicate fibrils branching freely, producing a very extensive root system. The new branches arise in two lines corresponding to the ends of the xylem plate in the diarch bundle. A longitudinal section of the rhizome, such as is diagrammatically represented in fig. 17, and a portion of which is shown in detail in fig. 18, indicates the endogenous origin of the root. VAN TIEGHEM, who has made an exhaustive study of the subject, states that they always arise from an endodermal cell.

The root apex is protected by a root cap, and also in its younger stages by the scales of the rhizome. This latter condition is represented in fig. 19. The root is formed by the cutting off of segments from a three-sided pyramidal apical cell (figs. 17-20). From the segments cut off parallel to the two lateral faces the tissues of the root arise. Segments cut off parallel to the remaining face are formative of the root tissue in part, but mainly of the root cap. The outer cells of the root cap are continually being worn away, and as continually being renewed by division of segments from the apical cell (fig. 20). The xylem first makes its appearance as two groups of tissue which appear simultaneously as the foci of the section (fig. 21). The elements are very small and have spiral and annular thickening on their walls. These protoxylem groups are differentiated long before any of the other elements. The remainder of the tissue at this stage is thin walled, and shows no marked characters, excepting that the endodermis even at this stage is well defined. The cortex also is composed of thin walled cells.

A curious feature of this form is that no definite root hairs are developed. Some of the epidermal cells may show short protuber-

ances, but there do not seem to be any normal, well defined root hairs. In the absence of these it follows that absorption must take place in one of two ways; either mycorrhiza may be present or the epidermal cells themselves may be especially adapted to absorb soil water by osmosis. In the absence of the former, it follows that the latter must take place. This perhaps may be a special adaptation under which the plant grows; root hairs would readily perish in the dry soil. When the water is available it must be absorbed rapidly, so that the necessity for the formation of root hairs, first of all, would seem to be a drawback to the plant; hence it seems feasible that water is absorbed more rapidly by osmosis through the thin walled epidermal cells.

About 1 mm. behind the apex, the epidermis and cortex become transformed into sclerenchyma. The root is diarch, the two protoxylem groups occurring at the ends of the xylem plate. The metaxylem elements are few, but very large; they have scalariform thickening laid down on their walls of the same nature as that in the stem and petiole. The phloem immediately surrounds the xylem with a little parenchyma intervening. The sieve tubes are few and do not form a continuous ring around the xylem, the phloem elements being absent in the vicinity of the protoxylem groups (fig. 22). Surrounding this is a pericycle, the cells of which are densely granular, as in other parts of the plant. The endodermis is not always to be seen in the older portions of the root, but where it does occur it is usually dark brown, with thick cell walls. When the root dries, however, the endodermis splits, and the vascular system becomes separated from the ground tissue.

GWYNNE-VAUGHAN (6) claims that the ontogeny of the vascular system as a whole is often repeated, although more or less imperfectly, in the development of the lateral shoots. In all the Polyodiaceae which he has examined, the root stele was invariably diarch. *C. tenuifolia* lends additional support to this view.

SORUS

The sori are developed later on the ordinary leaves. They are superficial on the lower surface of the leaf, and bear a close relation to the veins, usually forming above the free end of the small veins. They are protected merely by the inrolled margins of the leaf.

Fig. 23 represents a section through a soral region, and indicates that the receptacle is wide but flat, and from it numerous sporangia arise, all of different ages, younger sporangia interpolated between those already matured. Protecting these is the inrolled margin of the leaf, which may be termed an indusium, since it protects the sori. From its mode of origin and its structure, however, it is nothing more than the result of the outgrowth and thinning out of the margin of the leaf, which has become specialized for protective purposes.

The vascular strands run to the point immediately below the surface of the sorus, and then widen into a considerable mass of tracheids, surrounded by a layer of parenchyma, and limited by an endodermis.

The mode of development of the sporangia conforms with that usually found in the other *Mixtae* (figs. 24-28). The growth of the sporangium may be traced to a single epidermal cell (fig. 24). This cell protrudes above the surface of the epidermis, and is divided by a transverse wall level with the surface of the receptacle into a basal or stalk cell, and an upper cell which forms the sporangium. This BOWER considers to be the simplest segmentation of all. The basal cell takes no further part in the formation of the sporangium. The outer cell is divided by a strongly inclined wall which meets the basal wall, and this is usually followed by two others, so as to inclose a three-sided cell. From this a number of cells are cut off which form the three rows of cells seen in the stalk. GOEBEL was of the opinion that the stalk was separated from the capsule and formed from the basal cell, but the observations of various investigators on a large number of genera have proved this to be erroneous. The cells of the stalk undergo no further longitudinal divisions, so that it remains permanently composed of three or four rows of cells.

A periclinal wall is formed in the sporangium proper; thus a central cell and the wall cells are differentiated. The wall cells undergo no further periclinal divisions, but repeated radial divisions take place, so that the wall remains permanently one layer thick (fig. 25). Fig. 26 represents a still further advance in development. Here the sporangium has enlarged considerably, the contents of the

central cell (the archesporium) have become densely granular, and it has undergone periclinal division, so that a single-layered tapetum has been cut off. The cells of the tapetum undergo another periclinal division, and a two-layered tapetum is formed. This is constant and never varies. The tapetal cells undergo further divisions by radial walls. Fig. 27 indicates that a two-layered tapetum has been formed, and the archesporium has been divided into two cells by a vertical wall, which nevertheless is inclined to be oblique. In every instance observed this wall was vertical or oblique, but never transverse. A wall is then formed at right angles to this, dividing the archesporium into a quadrant of cells (fig. 28). Further transverse divisions take place in each cell, as a result of which an octant of cells is formed. The cells of the tapetum and archesporium have dense cytoplasm and large nuclei.

The next stage obtained was that in which the tetrads had been formed and the spores had separated. Unfortunately no stages between these could be obtained, but from similarity in other details it would seem probable that reduction division occurs in much the same manner as in the other Polypodiaceae. At maturity the cells of the tapetum disappear, the spores separate and become rounded, while the wall cells become organized into various parts. The number of spores is comparatively small, and this is characteristic of the Mixtae. The spore possesses several walls, the outer or perinium of which is usually brown and folded. The spore contents consist of one large nucelus and densely granular cytoplasm (fig. 29).

As in the other Mixtae, the annulus is vertical and interrupted by the stalk. Its cells have thickened walls, but the thickening does not occur on the outer part of the wall, which remains thin. The cells of the annulus which are situated near the stomium are larger than those occurring farther away. The other cells of the sporangial wall have thin cell walls.

The details of the development of the sporangium coincide very closely with those described for other members of the Mixtae. BOWER (1) considers that "reduction of the indusium has come along more than one line of descent. One such probable line might be traced from the Cyatheaceae with a two-sided indusium to *Hemitelia*, where it is one-sided. This has been responsible for the

exiguous, fimbriated indusium of *Woodsia* and *Hypoderris*. From this it is but a slight step to forms of the comprehensive genus *Polypodium*, where, with similarity of superficial sorus, the indusium is absent." The absence of an indusium in *Cheilanthes* has probably come along some such line of descent.

Conclusion

A study of the anatomy of *Cheilanthes tenuifolia* shows it to be a very interesting type, since in every part it reveals transitional characters. MARSH has shown that in *Cheilanthes* there exist types which are solenostelic, and others which are intermediate between solenostely and dictyostely. *C. tenuifolia* is a true dictyostelic type. Thus in the one genus the gradual transition from solenostely to dictyostely may easily be traced. Although dictyostely does occur, it is a very simple form of this type of anatomy, not comparable in complexity with the ultimate state of dictyostely which has been reached in the vast majority of the Mixtae. These considerations would place the genus among the early types.

The leaf trace and petiolar anatomy also tend to support this view. The leaf trace is single, and of a type characteristic of the solenostele; hence, although dictyostely does occur, it has just evolved, and is little removed from solenostelic types. In *Adiantum* the leaf trace is double, so that in this respect it is more advanced than *Cheilanthes*. The anatomy of the petiole is also simple. The vascular bundle is single, showing tendencies toward doubling, but nevertheless its structure is that characteristic of solenostelic types. GWYNNE-VAUGHAN claims that in stems with advanced dictyostelic structure the petiolar bundle is broken up into two or more portions, although where the structure is little removed from solenostely a single strand is often found. This is precisely what occurs in *C. tenuifolia*.

The leaf does not show reticulate venation, which is held to be a phyletic advance in forms in which it occurs.

ENGLER and PRANTL (4) classify the Pterideae into four sub-orders (Gymnogramniaeae, Cheilanthineae, Adiantineae, and Pteridineae). This position seems to be quite in accordance with the evidence of the present investigation. The anatomy proves it

to be of a simpler type than that which occurs in *Adiantum*, while *Pteris* has the most complicated leaf trace and dictyostely of all. There is a close relation between apical segmentation and development of the sporangium in all these types.

Cheilanthes is transitional between solenostelic and dictyostelic types of the Mixtae, between the simpler and the more complex. *C. Fendleri* is a true solenostele; the leaf gaps are long, but fail to overlap. *C. gracillima* is a true solenostele for the greater part of its course, but sometimes extra leaves are inserted. In *C. Lendgeri*, a type described by GWYNNE-VAUGHAN, each leaf gap is closed before the next leaf trace arises, so far as the phloem, endodermis, and pericycle are concerned, but the leaf gap in the xylem remains open; hence they overlap. In *C. lanuginosa* the stem is a dictyostele for the greater part, although sometimes the section is a perfect ring. *C. persica* and *C. tenuifolia* are true dictyostelic types. Right through this series, however, the leaf trace does not advance, but remains in the condition typical for a solenostele.

Every part of the plant shows adaptations to the xerophilous habitat. The subcoriaceous texture, the inrolled margins of the leaves (which, as well as serving as a protection for the sori, serve to control excessive transpiration from the stomata), stomata confined to the lower surface, all indicate well marked adaptations. The phloem is very much reduced in every part of the plant, hardly ever consisting of more than a single layer of sieve tubes. The pericycle is abnormally developed, and its cells are stored with reserve food, so that in this respect it serves as a storage parenchyma. The xylem parenchyma has also reserve food supplies. The cortex of stem, petiole, and root is highly sclerized.

From every standpoint *C. tenuifolia* proves to be a very interesting type, on account of its well marked xerophily, exceptional among ferns, and its characters intermediate between solenostely and dictyostely.

Summary

1. The apex of the rhizome is occupied by a tetrahedral apical cell.
2. The stelar anatomy of the stem is dictyostelic. The xylem is broken by wood parenchyma. There is no protoxylem present,

the first elements to be lignified being large, scalariform tracheids.

3. The amount of phloem present in the stem is small, consisting of a single row of sieve tubes surrounding the xylem, the amount of external being equal to the amount of internal phloem.

4. The pericycle is well developed, and has reserve food within it.

5. The apex of the leaf is occupied by a wedge-shaped apical cell.

6. The vascular strand of the petiole is single and concentric.

7. The petiole may be derived from types with triarch, mesarch protoxylem, described by SINNOTT. The medium protoxylem group moves its position, becomes endarch, and finally completely detached.

8. The leaf has the stomata localized to the lower surface, protected by the inrolled margins of the leaf alone. The cuticle and palisade tissue are well developed, while the lacunar tissue is reduced.

9. The root is endogenous in origin, and is formed by segments cut off from the three-sided apical cell.

10. The root is diarch, new branches arising in two lines corresponding to the ends of the xylem plates. The phloem is poorly developed, while the pericycle is well developed.

11. The sori are marginal, and are protected by the inrolled margins of the leaf; no true indusium is present.

12. The development of the sporangia corresponds mainly with that found in the other *Mixtae*.

13. The mature sporangium has a vertical annulus interrupted by the stalk.

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EXPLANATION OF PLATES VIII-X

FIG. 1.—Longitudinal section of apex of stem, showing cleavage of apical cell which will apparently give rise to dichotomous branching; $\times 202$.

FIG. 2.—Longitudinal section of apex of stem, showing tetrahedral apical cell and segmentation of same; scales present protecting apex; $\times 202$.

FIG. 3.—Same as preceding; $\times 460$.

FIG. 4.—Transverse section of stem made near apex; stelar anatomy dictyostelic; root and leaf traces being given off; cortex and pith not yet sclerized; $\times 25$.

FIG. 5.—Transverse section of meristele of dictyostele; xylem seen in center surrounded by phloem and pericycle; $\times 45$.

FIG. 6.—Transverse section of portion of meristele, showing nature of xylem, internal and external phloem, and a few of cells of pericycle; $\times 202$.

FIG. 7.—Longitudinal section of rhizome, showing xylem, phloem, pericycle, and endodermis; $\times 112$.

FIG. 8.—Longitudinal section of apex of leaf, showing wedge-shaped apical cell and segmentation from this; $\times 202$.

FIG. 9.—Transverse section of petiole, showing single vascular strand; $\times 45$.

FIG. 10.—Transverse section of portion of vascular strand of petiole, showing median protoxylem group dividing unligified metaxylem elements into two masses; other protoxylem groups endarch; $\times 112$.

FIG. 11.—Transverse section of vascular strand of petiole made further up on older stem; metaxylem here is single continuous mass; medium protoxylem group has become detached; $\times 112$.

FIG. 12.—Transverse section of portion of petiolar strand, showing nature of xylem, external and internal phloem, and a few of pericycle cells; $\times 202$.

FIG. 13.—Longitudinal section of vascular strand of petiole, showing nature of metaxylem and phloem; $\times 112$.

FIG. 14.—Transverse section of pinnule, showing upper and lower epidermis, stomata confined to lower surface, palisade parenchyma, and lacunar tissue and vascular bundle; $\times 112$.

FIG. 15.—Surface section of lower epidermis, showing nature of stomata and convoluted outline of epidermal cells; $\times 112$.

FIG. 16.—Transverse section of vascular bundle of pinnule; endodermis well marked and surrounds bundle; xylem surrounded by phloem and pericycle; $\times 202$.

FIG. 17.—Diagrammatic representation of longitudinal section of apex of stem, showing endogenous origin of root, also apex of stem and petiole; $\times 25$.

FIG. 18.—Portion of longitudinal section of apex of the stem, showing endogenous origin of root, also apex of root and petiole; $\times 112$.

FIG. 19.—Longitudinal section of root apex just emerging from stem, and still surrounded by scales of latter; $\times 202$.

FIG. 20.—Longitudinal section of root apex, showing apical cell of root, root cap, and root tissue; $\times 112$.

FIG. 21.—Transverse section of young root, showing two protoxylem groups already lignified; remainder of tissue thin walled; endodermis well marked; $\times 45$.

FIG. 22.—Transverse section of vascular bundle of root, showing diarch nature, also nature of xylem, phloem, and pericycle; $\times 112$.

FIG. 23.—Section through soral region, showing longitudinal section of vascular commissure, inrolled margin of leaf, and sporangia; $\times 112$.

FIG. 24.—Longitudinal section of young sporangium, showing development from single epidermal cell, which possesses very large nucleus; $\times 202$.

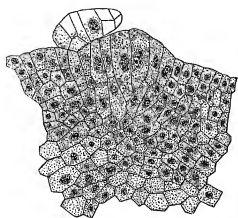
FIG. 25.—Longitudinal section of sporangium in which wall cells and stalk have been cut off, and archesporium consists of single cell; $\times 202$.

FIG. 26.—Further development of preceding; single-layered tapetum cut off; $\times 202$.

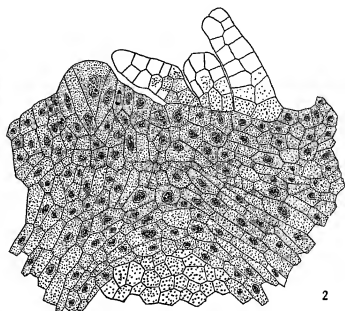
FIG. 27.—Transverse section of pinnule, showing transverse section of vascular commissure to sorus; further advance in development of sporangium also presented; tapetum two-layered; archesporial cell divided by slightly inclined vertical wall; $\times 112$.

FIG. 28.—Longitudinal section of sporangium still further developed; each of two cells shown in previous diagram has undergone division by transverse wall, so that quadrant of cells is formed; $\times 202$.

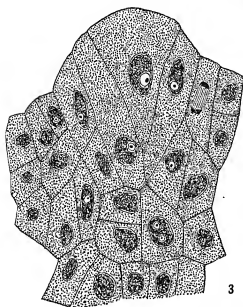
FIG. 29.—Section of spore, showing folded outer coat, large nucleus, and granular cytoplasm; $\times 202$.



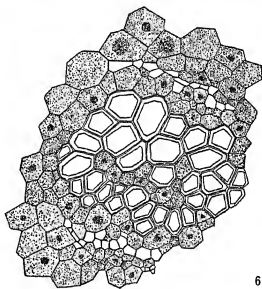
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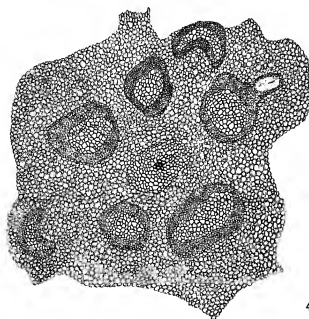
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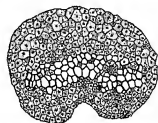
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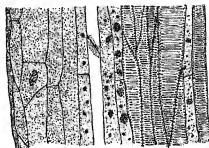
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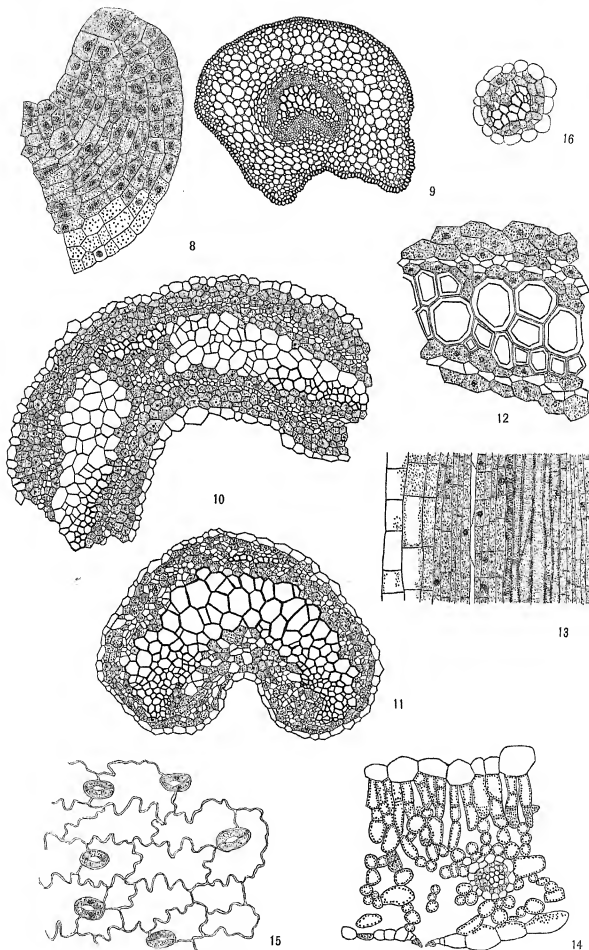


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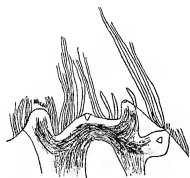


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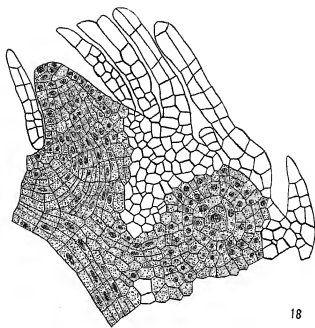




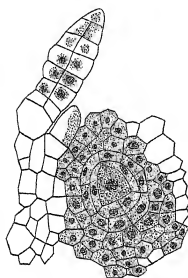




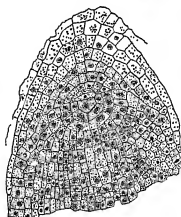
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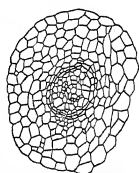
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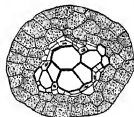
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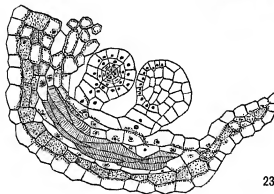
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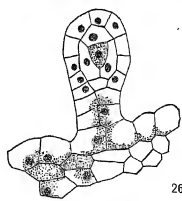
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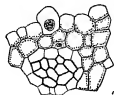
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EFFECT OF SIZE OF SEED ON PLANT PRODUCTION WITH SPECIAL REFERENCE TO RADISH

N. A. ROTUNNO

(WITH FOUR FIGURES)

Introduction

The effect of the size of seed on plant production, that is, crop yield, is an old problem. The literature reveals many references to the subject, together with varying results and differing opinions. In many cases the conclusions drawn are based on insufficient data when compared with the standards set by present day investigation. The methods employed by the numerous investigators have differed widely, consequently many of the results are not comparable.

The major part of the work has been done with cereals, especially wheat and oats. In the selection of experimental material for this work the writer gave consideration to those plants of which several crops could be grown in the greenhouse in one season. *Raphanus sativus* was selected as fulfilling this requirement. Very little work has been done with the radish, and the investigators have quite uniformly concluded that the large seed is superior to the small seed. The number of seeds used in most cases was comparatively small, and the weights of the resultant crops were usually taken en masse.

After preliminary experiments, it seemed desirable to obtain data with reference to the following questions: (1) Are weights of various sizes of seed comparable in different varieties? (2) Is there a difference in the percentage of germination between the large, medium, and small seed? (3) Which size of seed produces the heaviest roots? (4) Which size of seed produces the most marketable roots? (5) Are the results obtained with one variety true of another? (6) Is it profitable commercially to screen seed, and which size or sizes should be rejected if any? (7) Is there a correlation between the weight of the plant and the size of the root?

Historical

Among the earliest investigators who believed that the large seed was superior to the small was GALLOWAY (7), who worked with radish, using a total of 400 to 500 seeds. HICKS and DABNEY (12) reached the same conclusion on experiments with vetch, radish, rye, kaffir corn, and oats, using from 50 to 100 seeds of the large, medium, and small sizes for each crop. Since the number of seeds they used was small, and the experiments continued only for 24 days with radishes, 15 for vetch, 39, 23, and 23 for kaffir corn, rye, and oats respectively, and since in that time none of the crops tested had matured, the conclusions drawn are not entirely justified.

SHAMEL (18), working with tobacco, but making no statement as to the number of seeds used, finds that "careful comparative tests of light and heavy seed have proved that the best developed and the more vigorous plants are always produced by the large, heavy seed, while the small, light seed produces small, irregular, and undesirable plants."

EISENMENGER (5), experimenting with seeds of spruce, Scotch and Austrian pine, found that the large seeds germinated in shorter time and reached the period of germination earlier than small seeds, but that dark seeds gave a higher percentage of germination than light colored ones. SNYDER (19), in discussing light and heavy seeds of barley, wheat, and oats with particular reference to their composition, deduces from his observations that large seed is superior to small, or "that the farmer is repaid for using heavy weight seed." However, no actual data were presented as to tests on which he based his conclusion.

BURNETT (2), after extensive experiments on oats, using a large number of seeds of twenty varieties, states that "the figures tend to show that the heavy and light seeds were about equal, pound for pound, but not seed for seed, nor measure for measure."

HAYS (10), commenting on the results obtained from one test with oats, found that the heavy seeds produced more than the light. CUMMINGS (3), in discussing this subject after working with a large number of truck crops, made this general statement: "a distinct advantage follows the use of large seed or heavy seed." In the

same paper, with especial reference to the radish, with which he carried on sixteen different trials extending over a period of two years on two varieties, he states that large seed is superior to small. Not all the data of these trials were presented in this paper. In those recorded, CUMMINGS used in many cases only a small number of seeds, but in one case several thousands were used. The weighings were evidently taken en masse, and the comparisons made only between large and small seeds, although medium sized seeds were used in a few trials.

KIESSELBACH and HELM (13), working on a large scale with wheat for a period of ten years, conclude that "in general, results seem to indicate that when space is planted to permit maximum development a higher individual plant yield is obtained from large than from small seed."

Another group of investigators maintain that while the size of seed is an important factor, the origin and strain of the seed are of more importance than the size. Thus FINDLAY (6) found in his work with oats, barley, turnip, and red clover that in a single sample of seed the large produced more than the small, but in different samples of the same seed the strain and origin are of more importance than the size.

A third group of investigators believe that medium sized seed is superior to large and small. Among these are HICKMAN (11), who worked with three varieties of wheat, and used "selected seed consisting of the largest grains, the second grade the best of the wheat that passed through in screening out the first grade, and the unscreened seed, the entire lot as it came from the thresher," found that the second grade gave the highest yield in bushels per acre and in weight per bushel over large or unscreened seed in experiments carried on for a period of nine years. HARRIS (8), who experimented with *Phaseolus vulgaris*, concludes as follows: "In short, both large and small seed are less capable of developing into fertile plants than those which do not deviate so widely above or below the type." In a later paper (9) he states that "as the weight of the seed increases the time for germination becomes longer."

A fourth group of investigators are undecided in their opinions as to which size is best. Thus MONTGOMERY (15) finds "that after

eight years of continuous selection by the fanning mill, it was not possible by careful examination to note the difference in either the quality or quantity of the crop produced from the light and heavy seed." In a later paper (16), basing his conclusions on the results obtained on yield from one hundred plants of "large or plump" and "small or undeveloped" seed of both wheat and oats, he states that "small seed were at a marked disadvantage."

WILLIAMS (21), working with winter wheat, using twelve 6.5 inch pots planted with hand-sorted seed of large and small grains, and planting eight grains in each pot, found that the small seed produced more grain than the large seed. In drawing his conclusions, he says "the selection of grains, whether by means of the fanning mill or by hand, does not seem to promise anything in the way of permanent improvement in wheat in normal seasons." Later, in a paper on oats (20) in which the work was carried on for seven years, and evidently working with a large number of seeds, he finds that the large seed produced four bushels more per acre than small seed.

WILLIAMS and WELTON (22), using first, second, and third grade seeds of wheat and planting them at uniform rates on an extensive scale, conclude that when heavy and light seeds are separated by the fanning mill method there is not much hope of ever or even temporarily improving the yield of wheat; but with their work with oats (23), which was also done on a very large scale, they find large seed superior to small.

KIESSELBACH and RATCLIFF (14), experimenting with American Banner oats, using $1/30$ to $1/15$ acre plots, find that "as an eight year average the light seed has yielded 1.44 bushels more than the heavy." In the same paper, however, using the Kherson variety, they report that the large gave better results than the small seed. NIELSEN (17), after working with rye for ten years and using from 181.6 to 207.1 pounds of seed per acre, states that "in spite of the great difference in size of the seed the increase in the yield from the large seed was only slight."

It is evident that there are decided differences of opinion as to which size of seed produces the best results. It seemed logical,

therefore, to investigate further, using a large number of seeds, and growing the crops at different seasons of the year.

Materials and methods

The work here described was done in greenhouse benches 4 feet wide and 6 inches deep (fig. 1). Temperature and moisture conditions were kept as near the ideal as possible, although the former fluctuated at times. Before each sowing, the soil, a well prepared greenhouse soil, was spaded, raked, and prepared for planting. Rows were marked off 6 inches apart and half an inch deep.

In the first three series of plantings the seeds were divided into two groups only, large and small. The large or heavy seeds may

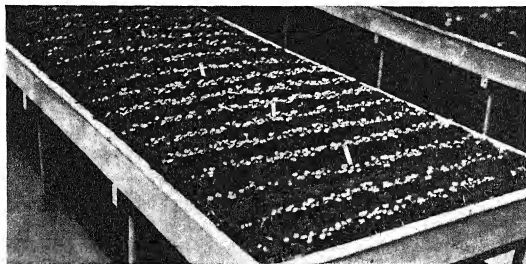


FIG. 1.—Greenhouse bench, showing how crops were grown; Scarlet Globe seedlings from small, medium, and large seeds.

be described as those which did not pass through a 12 mesh sieve. The small or light seeds are those which did pass through the sieve. Later the seeds were divided into three sizes, large, medium, and small. In these cases the large or heavy seeds were those which did not pass through a "chess" sieve, the openings of which were 2.1×12.7 mm.; the medium or middle weight seeds were those which did pass through this, but which would not pass through a 12 mesh sieve; and the small seeds were those which passed through the 12 mesh sieve and were the lightest of all.

This method of sorting gives a very distinct demarcation between the three sizes. Different methods have been used by other experimenters, the commonest being the fanning mill method, in which the light seeds are separated from the heavy seed. Another means of separating is the water method, in which the large seeds sink first. A modification of this is the use of liquids having various specific gravities. The writer believes the screen method to be superior to the others, in that it is more rapid and reliable, gives wide variations in sizes, and is more practical.

The varieties used in this work were the "Button Type" radishes suitable for forcing. The Scarlet Globe was used in the first trials because of its popularity locally for forcing. In later experiments, from one to five plantings were made of each of the following: Vick's Crimson Giant forcing, Burpee's Hailstone, Burpee's Scarlet Button, and Burpee's Rapid Red.

A total of twelve series was made. The seeds were sown about one inch apart, or 50 seeds in each row, which ran the width of the bench. Germination records were kept in all cases by daily notations which began with the appearance of the first seedling above ground.

When the majority of the plants had reached marketable condition, they were pulled, washed, and the surplus water allowed to run off. First the weight of the whole plant was recorded, and then the root was cut from the plant and weighed. These two weights were kept together for future references. The roots designated as salable were those which weighed at least 3 gm., and which had the characteristics of the variety they represented. The weights were taken to 0.01 gm., using a "Cenco" triple beam balance which is sensitive to 0.01 gm.

Records for each series were kept as follows: (1) average weight of 100 seeds of the large, medium, and small of each variety; (2) germination percentage; (3) weight of plant; (4) weight of roots from each plant; (5) average weight of the plants; (6) average weight of the roots; (7) biometric tabulations determining correlation between the weight of the plants and the weight of roots (in six series only); (8) number and percentage of salable plants from each size of seed. Records were kept of the date of planting, date

of harvesting, and the number of seeds of each size planted in each series. The data are given in table I.

TABLE I

SERIES	VARIETY	DATE OF PLANTING	NO. OF SEEDS PLANTED			DATE OF HARVEST	NO. OF GROWING DAYS
			Large	Medium	Small		
I*...	Scarlet Globe	2/ 2/22	100	100	3/13/22	40
II....	Scarlet Globe	3/16/22	300	200	4/21/22	36
III....	Crimson Giant forcing	3/16/22	200	200	4/24/22	39
IV....	Scarlet Globe	10/15/22	150	150	150	12/19/22	65
V....	Scarlet Globe	10/15/22	200	200	150	11/ 7/22	23
VI....	Crimson Giant forcing	10/15/22	150	150	150	11/ 7/22	23
VII....	Hailstone	1/26/23	150	150	150	3/16/23	49
VIII....	Crimson Giant forcing	3/ 1/23	200	200	200	4/ 9/23	39
IX....	Scarlet Globe	3/ 1/23	150	150	150	4/ 5/23	35
X....	Rapid Red	3/16/23	200	200	200	4/22/23	37
XI....	Hailstone	3/17/23	100	100	100	4/22/23	36
XII....	Scarlet Button	3/17/23	100	100	100	4/23/23	37

* In series I no count was kept as to the number of seeds sown. The plants were thinned to 100 of each from the large and small seeds and in each case the best plants of the lot were left. In all the other series, however, the exact count of the seeds was recorded in each case.

Results

RELATION OF SIZE TO WEIGHT

As previously described, the seeds were separated into the groups large, medium, and small, and ten samples of 100 seeds of each size were counted, weighed, and the average weight of 100 seeds computed. This was done with all the varieties discussed in this

TABLE II
AVERAGE WEIGHT IN GM. OF 100 SEEDS COMPUTED FROM
10 SAMPLES

Variety	Large	Medium	Small
Scarlet Globe.....	1.06	0.74	0.49
Crimson Giant forcing.....	1.09	0.79	0.59
Hailstone.....	0.90	0.74	0.55
Rapid Red.....	0.93	0.61	0.42
Scarlet Button.....	0.95	0.69	0.51

paper. The results obtained are given in table II. A very distinct direct relation of size to weight seems to exist in all the varieties. The seed designated as medium is about two-thirds the weight of

the large, and the small seed about two-thirds the weight of the medium. The relative sizes of the seeds are shown in fig. 2.

GERMINATION

Much has been written about the difference in germination between the large and small seed, and in almost all cases it is claimed

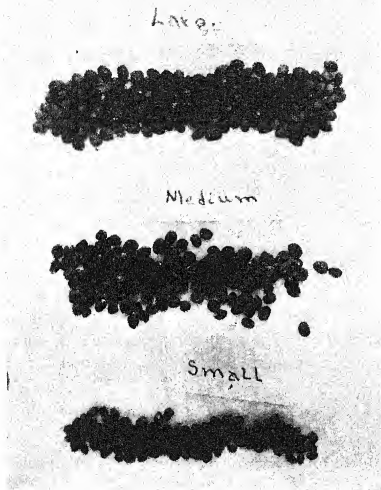


FIG. 2.—Seed of Scarlet Globe variety, showing relative sizes of large, medium, and small seeds; slightly reduced.

that the large seed has a decidedly higher percentage of germination than the small. Since some differences of opinion were noted in reviewing the literature, germination records were kept to substantiate or disprove the general opinion held on this point. Table III presents the data obtained in these tests.

From Table III it is evident that there is a decided variation in the percentage of germination between the various sizes of seeds. Considering the series singly, there are cases in which the large have an advantage over the medium and small, and in other cases the medium or small show an advantage over the others. As will be noted, the large seed of the Scarlet Globe variety shows a slightly higher percentage of germination over the medium and small seed. In the Rapid Red variety the small seed has a marked advantage over the large and medium, whereas in the Crimson Giant variety

TABLE III

SERIES	VARIETY	No. OF SEEDS USED			No. GERMINATED			PERCENTAGE GERMINATION		
		Large	Medium	Small	Large	Medium	Small	Large	Medium	Small
II.	Scarlet Globe	300	200	246	143	82.0	71.5
IV.	Scarlet Globe	150	150	150	127	111	110	84.6	74.0	74.0
V.	Scarlet Globe	200	200	150	133	146	106	66.5	73.0	70.0
IX.	Scarlet Globe	150	150	150	139	132	114	90.2	89.0	76.0
III.	Crimson Giant	200	200	143	153	71.5	76.5
VI.	Crimson Giant	150	150	150	121	103	123	80.0	70.0	80.2
VIII.	Crimson Giant	200	200	200	129	117	124	64.5	58.5	62.0
VII.	Hailstone	150	150	150	120	131	86	80.0	87.3	57.3
XI.	Hailstone	100	100	100	70	72	83	70.0	72.0	83.0
X.	Rapid Red	200	200	200	72	73	104	36.0	36.5	52.0
XII.	Scarlet Button	100	100	100	28	27	24	28.0	27.0	24.0
Total.	1900	1400	1750
Average.	68.48	70.03	66.50

the small seed shows a slight increase over the large and medium seed when averaged. In the Hailstone variety, when the percentages are averaged, the medium shows a decidedly higher percentage of germination than either the large or small, while in the Scarlet Button variety the large and medium size germinate about the same, and both show slightly better germination than the small seed. The average for each of these sizes for all the varieties, taken as a whole, indicates that the difference between them is slight and within limits of experimental error.

CORRELATION BETWEEN WEIGHT OF PLANTS AND ROOTS

So far as could be ascertained, no one has reported as to whether a correlation exists between the weight of the plant and the weight

TABLE IV
COMPARISON OF RESULTS OF 12 SERIES OF PLANTINGS

Series	No. harvested	Total weight in gm.	Average weight of plants	Weight roots	Average weight	No. salable	Percentage salable
I*: <i>Scarlet Globe</i>							
Large.....	100	829.90	8.29	312.50	3.1200	41	41.0
Small.....	100	906.20	9.06	396.40	3.9600	55	55.0
II: <i>Scarlet Globe</i>							
Large.....	246	1639.25	6.60	506.10	2.050	64	26.0
Small.....	143	821.00	5.70	202.80	1.600	24	16.5
III: <i>Crimson Giant forcing</i>							
Large.....	143	1730.11	12.10	525.90	3.600	55	38.4
Small.....	153	1880.67	12.30	572.70	3.700	68	44.4
IV: <i>Scarlet Globe</i>							
Large.....	127	720.90	5.60	206.20	1.710	23	18.0
Medium.....	111	768.78	6.90	275.12	2.480	40	41.0
Small.....	110	566.50	5.10	151.90	1.380	16	14.0
V: <i>Scarlet Globe (3 weeks old)</i>							
Large.....	133	122.85	0.91	13.83	0.100
Medium.....	146	183.96	1.26	22.75	0.160
Small.....	106	108.11	1.02	13.27	0.125
VI: <i>Crimson Giant forcing (3 weeks old)</i>							
Large.....	121	195.20	1.61	12.58	0.104
Medium.....	103	174.10	1.69	9.40	0.090
Small.....	123	185.00	1.50	13.83	0.112
VII: <i>Hailstone</i>							
Large.....	120	561.00	4.70	217.10	1.800	26	21.6
Medium.....	131	738.50	5.60	280.48	2.140	32	24.4
Small.....	86	263.08	3.05	101.16	1.170	9	10.4
VIII: <i>Crimson Giant forcing</i>							
Large.....	129	1442.74	11.17	386.53	3.040	45	34.9
Medium.....	117	1359.25	11.60	370.75	3.190	44	37.6
Small.....	124	1376.15	11.10	401.77	3.280	49	39.5
IX: <i>Scarlet Globe</i>							
Large.....	139†	1221.90	8.79	453.85	3.260	69	50.0
Medium.....	132	1072.28	8.12	435.47	3.300	75	56.7
Small.....	114	576.15	5.10	235.09	2.060	35	30.7
X: <i>Rapid Red</i>							
Large.....	72†	496.91	6.90	315.53	4.370	47	65.2
Medium.....	73	527.08	7.22	369.44	5.060	62	85.0
Small.....	104	403.39	3.88	240.65	2.310	38	36.5

* In series I only the best plants were selected and allowed to mature.

† Two plants in each of these series were off type, therefore are not included.

TABLE IV—*Continued*

Series	No. harvested	Total weight in gm.	Average weight of plants	Weight roots	Average weight	No. salable	Percentage salable
XI: <i>Hailstone</i>							
Large.....	70	327.35	4.67	165.81	2.360	25	35.7
Medium.....	72	454.89	6.31	215.48	2.990	39	54.1
Small.....	83	536.60	6.46	260.98	3.140	41	49.4
XII: <i>Scarlet Button</i>							
Large.....	28	176.27	6.29	91.29	3.26	11	39.3
Medium.....	27	148.30	5.49	76.65	2.83	12	41.5
Small.....	24	139.30	5.8	72.61	3.02	11	45.8

of the root in the radish. Since the individual weight of the plants and their roots had been recorded, it was thought advisable to determine whether or not a correlation existed between these two weights. Biometric tables were arranged using one gram classes. Thus all plants or roots weighing between one and two grams were placed in the 1.5 class, those weighing from four to five grams were placed in the 4.5 class, etc. Table V represents the results of the correlations from series 2, 3, 4, 5, and 6 for the large, medium, and small seeds.

TABLE V

VARIETY	SERIES	CORRELATION COEFFICIENTS FROM		
		Large	Medium	Small
Scarlet Globe.....	2	+0.91±0.01	+0.83±0.016
Crimson Giant forcing..	3	+0.89±0.01	+0.88±0.012
Scarlet Globe.....	4	+0.82±0.019	+0.74±0.025	+0.80±0.023
Scarlet Globe.....	5	+0.46±0.05	+0.80±0.02	+0.67±0.03
Crimson Giant forcing..	6	+0.59±0.04	+0.58±0.04	+0.67±0.03

Since it is generally agreed among biometricians (1) that, to be significant, the ratio of correlation should be at least six times the probable error, it appears that a definite correlation exists.

Discussion

In the majority of papers cited there are usually no data to indicate the difference in weight between the large, medium, and small seeds. It is possible that in tests where wheat is used the

small seed is the lightest in weight, but with oats the relation between size and weight may not hold, as it is possible that a large oat seed may not weigh as much as one which is smaller. Table II, however, indicates that with radish seeds a distinct relation exists between size and weight when screened, according to the methods previously described. The differences are so marked and distinct that they can justly be called "large, medium, and small" seeds, or "heavy, medium, and light" seeds. In other words, "large, medium, and small" as to size correspond with "heavy, medium, and light" as to weight.

With reference to the differences in percentage of germination between large, medium, and small seeds of radish, GALLOWAY (7) and CUMMINGS (3), basing their conclusions on results obtained from two varieties, say that large seeds give a higher percentage of germination than small seeds. Variety differences, however, have appeared in the present work. From the data presented in table III it appears that the varieties used in these experiments group themselves into three classes, as follows:

First, in the Scarlet Globe variety the large seeds give a higher percentage of germination than the small when only large and small seeds are used. When the seeds are divided into three groups, however, large, medium, and small, the large gives only a slightly higher percentage of germination than the medium, and both have an advantage over the small seeds. Likewise, with the Scarlet Button variety the large have a very slight advantage over the medium, and again both are superior to the small seeds.

In the second group are those varieties in which the medium seeds are superior to the large or small. Thus in the Hailstone variety, as an average for two trials, series VII and XI representing altogether 750 seeds, the medium seeds give the highest average percentage of germination.

In the third group, which includes the Crimson Giant forcing and the Rapid Red varieties, the small seeds quite uniformly give the best germination when the seeds are divided into large, medium, and small sizes. With the Crimson Giant forcing variety the small seeds germinate best, even though only large and small seeds are used (series III).

It is evident, therefore, that the percentage of germination of the large, medium, and small seeds differs with the variety. When the percentages are averaged for all the series, however, they are quite uniform, and no one size has a great advantage over the others. These results when taken as a whole do not agree with those obtained by previous investigators. Since, however, the number of seeds used is sufficiently large, and since more varieties are included in the present work than in any previous work on radishes, the results may be regarded as significant.

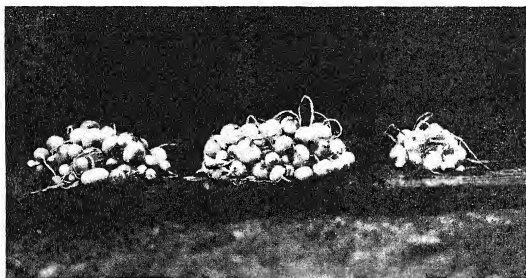


FIG. 3.—Roots from 150 seeds each of large, medium, and small seeds of Hailstone (reading from left to right).

In growing radishes commercially, the important part of the plant is the root. The smaller the tops and the larger the roots, within certain limits depending on the market and the variety, the better the sales. Of course, the larger the photosynthetic tissue the more rapid the growth, other things being equal. For this reason it was interesting to note which size produced the best product. A summary of the results obtained with the twelve plantings is shown in table IV. It will be seen that the results from the different sizes again group themselves according to variety.

In the one trial of the Scarlet Button, the large seeds produced a slightly heavier root than the medium or small seeds. With the Scarlet Globe variety (series II), when large and small seeds only

are used, the large produce a heavier root than the small, but when large, medium, and small seeds are used (series IV and IX), the medium seeds produce a decidedly heavier average root (2.89 gm.) than either the large (2.48 gm.) or the small (1.72 gm.).

In the two series of the Hailstone variety, one of the trials shows that there is a strong tendency for the medium seeds to produce the heaviest roots, while in a trial made later in the spring the small seeds produced the heaviest roots with the medium a close second. The average for the two series shows that the medium seeds produced the heaviest roots. In the several trials with the Crimson Giant forcing



FIG. 4.—Roots from 200 seeds each of large, medium, and small seeds of Crimson Giant forcing variety (reading from left to right).

variety, the plants from the small seeds in every case produced heavier average roots than the large or medium seeds. The Rapid Red variety seems to exhibit the same tendency as the Crimson Giant forcing.

It is evident, therefore, that there is no uniformity of results from seeds of the same size in different varieties, and therefore they do not conform to the results of previous investigators.

With regard to the percentage of salable roots produced from large, medium, and small seeds, there seems to be as much variation as with the other characters. The trials in which the Scarlet Globe variety was used show that when only large and small seeds are used, the large produce a higher percentage of salable roots

than the small (series II); but when the three sizes, large, medium, and small are used, the medium produce the highest percentage of salable roots. The same phenomena hold true with the Hailstone (fig. 3) and Rapid Red varieties. In the Crimson Giant forcing variety (fig. 4) the small seeds in every case produce the highest percentage of salable roots. The same is true of the one trial of the Scarlet Button variety.

Summary

This paper is to be regarded as a preliminary report on seed studies on the radish. Observations for a number of seasons and on a larger number of seeds should be made to settle definitely some of the phases of the subject touched on in this paper. From the data presented, however, the following conclusions seem justified:

1. The relative weights of the same sizes of seeds in different varieties differ widely, but the relative weights of the large, medium, and small seeds are approximately the same in all varieties.

2. The percentage of germination varies considerably with the large, medium, and small seeds. In some varieties the large seeds germinate best, in others the medium, and still others the small. When the average percentage of germination for the various sizes of the several varieties is computed, the difference between any of the sizes is negligible and within the limits of experimental error.

3. No definite statement can be made as to which size produces the heaviest root. In the majority of trials with the large, medium, and small seeds of the Scarlet Globe, Hailstone, and Rapid Red varieties the medium seeds gave the best results; with the Crimson Giant forcing variety the small seeds always produced the heaviest roots; and in the one trial of the Scarlet Button variety the large seeds produced the heaviest roots. When taken as a whole and averaged, the medium seeds produce the heaviest roots.

4. With reference to the percentage of salable roots, the medium seeds in every variety, except the Crimson Giant forcing, produce the highest percentage of salable roots.

5. The question as to why in one variety the small seeds should give the largest roots, and other varieties the large, and in still others the medium remains unanswered. Theories might be formu-

ated, but the first requisite should be additional dependable data.

6. Apparently there is a definite correlation between the weight of the plants and the weight of the roots.

7. From the data herein recorded it seems established that the results obtained with one variety are not necessarily comparable with another variety, as stated by GALLOWAY (7) and CUMMINGS (3).

The writer takes pleasure in extending thanks to Professors HENRY F. A. MEIER and C. C. CARPENTER for kindly suggestions and criticisms freely given throughout the progress of the work.

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APPLE TUFTS

C. C. CARPENTER

(WITH SIX FIGURES)

Introduction

Apple tufts are the white growths that appear on the carpel walls and seeds of some varieties of apples. They are found along fissures in the carpel walls, which generally run at right angles to the longitudinal axis of the fruit, and upon the seed as a white cotton-like growth. Under the microscope the strands appear separate, almost hyaline, and upon almost every cell wartlike protuberances are found in numbers (figs. 1, 2).

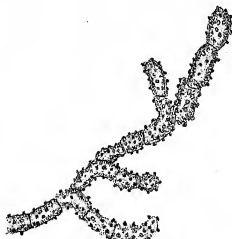


FIG. 1



FIG. 2

FIGS. 1, 2.—Fig. 1, typical tuft strand showing protuberances on cell walls and characteristic manner of branching; fig. 2, strand of tuft tissue showing manner of development; $\times 200$.

In the literature consulted, SORAUER (8) has given more attention to apple tufts, or "Wollstreifen" as he calls them, than any other investigator. He considers the tufts a non-parasitic apple disease, and states that it is more common and is produced in greater abundance in wet seasons, and is of the opinion that it enters the fruit through the calyx cavity at some time early in its development, and that it is a factor in causing apples to decay. He believes that

when it occurs the fruit must be used soon or the loss from decay will be great. His drawings show the characteristic protuberances on the two or three terminal cells of a tuft strand, and the presence of leucoplasts such as may be found in fresh material. SORAUER also shows detached areas of the thick celled carpel wall as is shown in figs. 3 and 4 of this paper. He describes in some detail the results secured by using different stains on tuft tissue.

BEACH (1) uses tufts as one of the characters in his apple descriptions, and states that they are found in many varieties. He offers no explanation as to their structure, composition, or origin. HEDRICK (5) mentions tufts repeatedly as a variety characteristic. They are shown in photographs in technical bulletins (6), but no mention is made of them in the text of the bulletins consulted. They are also shown in certain varieties in hand paintings by EMMONS (3).

In some varieties, such as Tallman, R. I. Greening, and Baldwin, tufts are nearly always present. In 319 specimens of Tallman, the writer found only two fruits, or less than 1 per cent, that did not show tufts. In R. I. Greening only one fruit in 146 did not show tufts. Jonathan, both New York and Northwestern grown, failed to develop tufts, although some apples were found that showed the characteristic fissure through the carpel wall along which tufts develop. Northern Spy is variable in regard to this character. Samples of this variety were taken from three rather widely separated locations, Syracuse, South Onondaga, and Geneva, which showed tufts as follows:

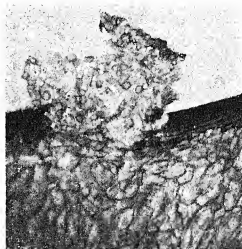


FIG. 3.—Tallman: cross-section showing detached portion of carpel wall dislocated by tuft tissue; $\times 100$.

	SYRACUSE	SOUTH ONONDAGA	GENEVA
Number of fruits.....	639	163	153
Percentage showing tufts.....	41.6	38.7	44.9

It seems that the presence of tufts in Northern Spy was fairly uniform under different environments and in different localities for

the year 1922. Whether this variety uniformly develops them in these percentages would have to be determined by counts over a period of years. This was a wet season in Central New York. According to SORAUER, one should expect a smaller percentage of specimens to show tufts in a dry year. Northern Spy is a good variety in which to study this phenomenon, although probably others are equally good.

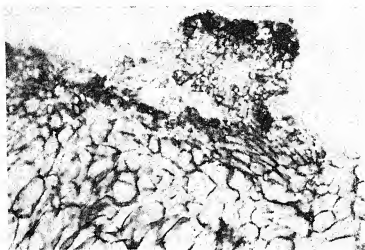


FIG. 4.—Newtown: longitudinal section through carpel fissure showing detached area of carpel wall dislocated by tuft; $\times 100$.

Correlation of tufts with other morphological characters

OPEN CORE.—In talking with orchardists and pomologists, the statement has been made a number of times that tufts are generally found in open core apples. Desiring to determine in regard to this, upon examination it was found that 215 of the varieties of apples in the *Cyclopedia of Hardy Fruits* were so described for core characters that correlation could be determined. In this publication the core was described as open, partly open, open or closed, closed or open, and closed. It was supposed that when varieties were recorded as being found with both open and closed cores, the commonest type was placed first in the description, and was arbitrarily so assumed for purposes of this calculation. On this assumption the following was secured:

TABLE I

	TUFTS ABSENT	TUFTS PRESENT
Closed core.....	71	65
Open core.....	45	34

In this four-fold table the alternative variates in both cases are unquestionably continuous. Testing first for goodness of "fit" (2), it was found that P was relatively small (0.000865); this being the case, the "tetrachoric r_t " was determined (4). For this table $r_t = -0.066 \pm 0.073$. This indicates that there is no correlation between openness of core and the phenomenon of tufts.

OPEN CALYX.—SORAUER is inclined to think that the fungus (as he calls it) which constitutes the tufts probably enters the apple through the calyx cavity at some time during the development of the fruit. Thinking that there might be some association or correlation between open type of calyx and tufts, the *Cyclopedia of Hardy Fruits* was again consulted, and 201 varieties were described definitely enough so that they could be tabulated for this correlation:

TABLE II

	TUFTS ABSENT	TUFTS PRESENT
Closed calyx.....	87	75
Open calyx.....	20	19

Calculating as described for table I, it was found that $P = 0.0053$, and that "tetrachoric r_t " = -0.139 ± 0.069 . This unquestionably indicates that there is no correlation between type of calyx and the presence of tufts.

ABORTIVE SEEDS.—It was thought that there might be some relation between tufts and abortive seed in an apple. From data secured by the writer, it is apparent that one of the factors which cause abortive seed is that when competition is strong enough between fruits, an apple will abort seeds something like a tree will drop fruit if the food supply is low or the tree is suffering from lack of moisture. If tufts are a fungal development, however, as stated by SORAUER, it seems logical to suppose that there might be some correlation between these phenomena.

Size of seeds in apples range from a plump mature seed down to a rudimentary type hardly discernible to the eye. In this paper "abortive seed" is one in which neither embryo nor endosperm has developed. In many seeds to determine this it was necessary to section the seed longitudinally. The seeds were cut in this way, since if only a small endosperm is developed it would be found at

the micropyle end of the seed. On this basis the data given in table III were secured.

In table III the x variate admits of only two divisions, which are certainly not discrete, as the amount of tufts is continuous from a maximum to the condition where no tufts are found. As one series consists of graduated measures and the other is dichotomous, the

TABLE III
CORRELATION BETWEEN ABORTIVE SEEDS AND TUFTS IN NORTHERN SPY;
NUMBER OF FRUITS, 483; COEFFICIENT OF CORRELATION
 $r = -0.277 \pm 0.054$

CONDITION	ABORTIVE SEED												TOTAL
	0	1	2	3	4	5	6	7	8	9	10	11	
Tufts.....	18	28	26	30	34	28	16	7	3	0	1	2	193
No tufts.....	48	62	60	49	24	28	10	3	3	1	2	0	290

bi-serial r formula, as given by KELLEY (7), is used to determine the amount of relationship between the two characters under discussion. Considering the size of the sample and the amount of correlation determined, it appears that there is no significant relation between these two characters.

Relation between tufts and amount of seed for size of apple

In cutting a large number of apples for seed counts, it became apparent that there was some relation between number of seeds and the presence of tufts. In Northern Spy particularly was this noticed. In this variety, if one found a large fruit with lower than the average number of seeds for this sized apple, tufts were nearly always present. The same sized apple with a normal number of seeds, or a small apple with the same number of seeds, as a rule would show no tufts. To get some basis for making a correlation calculation, the weight of the seed was divided by the weight of the apple, which would give a ratio between these characters for each fruit. This would give a low ratio for apples having heavy weight and few seeds, and small or large apples having many seeds would have a high ratio. This again gives an array in which one series consists of graduated measures and the other is dichotomous. These

data are presented in table IV. Noting the correlation coefficient obtained from this table, it is evident that there is a considerable degree of correlation between low seed content for the size of the apple and the presence of tufts. From this it would follow either

TABLE IV

RATIO BETWEEN SEED AND FRUIT WEIGHTS; NUMBER OF FRUITS, 465;
COEFFICIENT OF CORRELATION $r=0.505\pm0.046$

Condition	3	5	7	9	11	13	15	17	19	Total
Tufts.....	28	68	55	32	13	196
No tufts.....	7	42	91	62	39	12	9	3	4	269

that if an apple has few seeds for its weight it has a surplus of material which goes into the production of tufts, or that since tufts are found along carpel fissures, when an apple of certain varieties has few seeds the carpel walls are more likely to crack, thus permitting tufts to develop.

Pathological studies

Since SORAUER considered that tufts are a fungus, comprehensive studies using pathological technique were employed in studying this phenomenon in apples.¹ While several varieties of apples were examined with the idea of determining the nature of apple tufts, considering the number of named varieties a representative number was by no means studied. Varieties studied included Northern Spy, Tallman, Delicious, Newtown, Baldwin, R. I. Greening, Jonathan, Ortley, York Imperial, and Ben Davis, in this and the following phase of this paper.

Working on the assumption that tufts are a fungus, attempts were made to cause them to grow and fruit in the way that fungi are usually studied. Portions of the carpel wall containing tufts were removed from the core of the apple, and placed in tubes containing apple agar made from the same variety of apple from which the tuft studied was secured. Fifty tubes streaked in various ways as to depth, amount of tuft, etc., failed to cause the tufts to develop to the slightest observable degree. Also portions of apple in Petri and

¹ This phase of the work was carried on with the assistance of Dr. E. REED of the Department of Botany at Syracuse University.

Stender's preparation dishes were autoclaved, and tufts placed in and on this material refused to develop. Receptacles containing tufts were placed in an incubator and subjected to a temperature of 40° C. without effect. Part of these were then removed to a room with a temperature of 5° C. Samples were subjected to these conditions from three to fifteen days, hoping that the changes from one extreme to another would shock the assumed fungal growth and cause it to fruit.

No set or combination of conditions caused the tuft to develop to the slightest extent. While the tufts were thus being studied they were examined daily with the microscope, and it was uniformly noted that the tufts were the same color and would dry out in the same manner as the apple tissue under all the conditions mentioned. Special fungal stains such as alum eosin were used, and in all cases the tuft took the stain the same as did the apple tissue. These results lead to the conclusion that apple tufts are not a fungus. At the same time, the tuft being very similar to apple tissue, except as to form, makes an excellent medium for fungi that develop in apples, so that when any part of a fungus comes in contact with a mass of tufts, in all likelihood it will develop under suitable temperature conditions, and this might lead one to think that the tufts were masses of fungal tissue. Such confusion of tuft and fungal tissue may explain the conclusions of SORAUER on the subject.

Microscopic studies

Coincident with the pathological work, areas of carpel wall were selected containing tufts attached to apple tissue. These were removed from the apple core, killed, and fixed by using Flemming's solution. Following the usual processes some of the material was imbedded in paraffin, but owing to the difficulty of cutting the tough leathery carpel wall it was necessary to use celloidin as an imbedding material. Various stains were tried, such as alcoholic safranin, iodine, light green, iron alum, and alum eosin, but none gave results comparable with a light stain of Kleinenburg's haematoxylin. With this stain the apple and tuft tissue take on a clear purplish-blue color and the carpel wall remains unstained, giving a marked contrast between the different types of tissue. The tufts take the stain in

the same amounts as to color as the apple tissue just outside the carpel wall, indicating that they are identical with apple tissue and are not fungi or storage bodies, as might be indicated by the correlation of tufts and low ratio between weight of fruit and weight of seed previously mentioned. The sectioned and stained material shows clearly that the tufts are proliferations which develop from the pith tissue in some varieties when a fissure forms in a carpel wall (figs. 5, 6). Frequently areas and sometimes single cells of the carpel wall are separated when the wall cracks, and these may be dislocated and pushed out by the developing mass of tufts (figs. 3, 4).

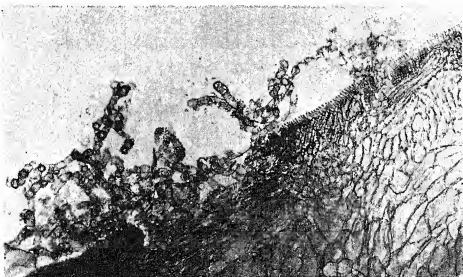


FIG. 5.—Newtown: cross-section showing tuft tissue through fissure in carpel wall and protuberances on strands of middle group; $\times 100$.

While tufts are found on seeds, the sections made failed to show that at any place they were more than superficially attached to the seed. It seems probable that as they develop they grow along the surface of the seed where it happens to be contiguous to a carpel fissure.

It was noticed that there was a slight difference in the tufts produced in the different varieties of apples. In Northern Spy the cells in the strands average $65\ \mu$ in length and about $20\ \mu$ in diameter. Tallman shows cells slightly less in diameter, but of about the same length. The chief difference noted is in the terminal cells. Fresh material of Northern Spy shows protuberances stalked and almost globular which are iridescent under the microscope. Tall-

man shows these protuberances, but they are not so well developed as to size and show no coloring. Fresh material shows round bodies in the cells, some protoplasmic streaming, and with an iodine stain nuclei are quite distinct in the terminal cells.

Whether any physiological conditions are a factor in the development of tufts, and whether they are of economic significance have not been determined yet by the writer. It seems that their presence

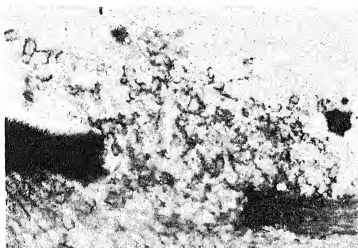


Fig. 6.—Newtown: cross-section of typical type of tuft; $\times 100$

is a varietal characteristic, however, and one would be justified in using them as a means of classification of apple varieties if desired.

Summary

1. Tufts in apples are not fungi, but are tissue proliferations developed in carpel fissures in certain varieties of apples.
2. Tufts are found in high percentages in some of our longest keeping varieties, and while they may be a factor in decay, apples containing them will store without decay for long periods.
3. Tufts are not correlated with open core, open calyx, or with abortive seeds.
4. In Northern Spy tufts develop when an apple has a low seed content for its size.
5. Tufts produced in different varieties of apples show slight differences.

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EFFECT OF LIGHT ON GROWTH OF EXCISED ROOT TIPS UNDER STERILE CONDITIONS¹

WILLIAM J. ROBBINS AND W. E. MANEVAL

(WITH TWO FIGURES)

Experiments on the growth of excised corn root tips under sterile conditions, reported in previous papers (2, 3, 4), showed that if the original excised root tip was grown in the dark for ten days or two weeks in Pfeffer's solution plus 2 per cent glucose, and its tip then cut off and transferred to a fresh solution at two-week intervals, the growth ceased in the third period. It was found that the addition of autolized yeast or peptone to the solution permitted the growth of the root tips to continue for as long as 4-6 periods in the dark. Even in the presence of autolized yeast or peptone, however, the rate of growth continually decreased as the periods passed, and eventually stopped. Attempts to improve conditions by increasing the concentration of autolized yeast showed that while 400 ppm of autolized yeast was somewhat more favorable than the other concentrations used, there was no marked difference between the effects of the various concentrations, and none of them permitted continued growth. TURNER (6) and KOTTE (1) have also grown excised root tips of higher plants under sterile conditions.

The specific problem dealt with in these investigations is the problem of defining the classes of materials which must be present, and the conditions that must exist, in order that the root cells will continue to grow. Do the cells of a corn root require something more than the mineral salts of Pfeffer's solution, oxygen, water, and glucose for their growth? The results of the experiments performed thus far and reported elsewhere could be interpreted as indicating that these materials are insufficient for the continued growth of excised roots, and that peptone or autolized yeast supply a part of the deficiency. Other interpretations of the data might

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be made, however, and other explanations offered for the cessation of growth to which attention has been called.

The present paper is the record of experiments in which the effect of a second factor favorable for the growth of excised corn roots is reported. This factor is light. Diffuse light favors the growth in Pfeffer's solution plus 2 per cent glucose of an excised root tip when its meristematic tissue is transferred to fresh solutions at intervals. In the presence of both light and autolized yeast, excised root tips of corn have been kept growing in Pfeffer's solution plus 2 per cent glucose for 149 days through ten periods of transfer.

In an earlier experiment, performed by the senior writer, and which has not been reported, the excised root tips of corn were grown under sterile conditions in culture flasks which were aerated by bubbling air through the solution continuously during the experiment. These flasks were placed in diffuse light to permit their attachment to a suction pump. It was noted that the growth of the root tips in both aerated and non-aerated cultures in the second growth period was superior to that in any of the experiments previously performed in the dark. An experiment was begun April 2, 1921, therefore, to test the effect of light and the combined effect of light and autolized yeast upon the growth of the excised root tips.

In experiment 13 the methods used were similar to those used in experiments previously reported. Root tips of Longfellow flint corn were grown under sterile conditions in 50 cc. of solution in 150 cc. Erlenmeyer flasks of Pyrex glass, a single root tip being placed in each flask. The solutions used consisted of the modified Pfeffer's solution plus 2 per cent glucose, and the same solution with 80 ppm or 400 ppm of autolized yeast added. The autolized yeast extract was prepared in essentially the same way as described earlier. Half of the flasks in each set were placed in diffuse light from a north window, and the other half were wrapped in black paper, covered with a black cloth, and placed in the same location. No method was available for controlling the temperature, which varied from 16° to 30° C. At two-week intervals the primary tip of each root was cut off and transferred to a fresh solution of the same composition as the one in which it had previously grown.

Three days after the root tips had been cut from the grains and transferred to the solutions, some of those in the light had developed anthocyan. Some of the roots in the light never developed anthocyan, however, and none of the roots in the dark developed this pigment. At the end of a week the distribution of anthocyan was very interesting. The heaviest development was in the older part of the root near the base, and around the points of emergence of the secondary roots. That portion of the root in which no secondary roots had yet developed showed red spots, indicating the points at which secondary roots were pushing through the cortex. The root cap cells also were red. The heaviest development of anthocyan occurred in the first period. There was apparently no positive correlation between the development of anthocyan and growth; in fact, the two roots which grew for the longest period contained no anthocyan.

During the first two weeks of growth there was little difference in gain in length (table I) between those roots which grew in the dark and those which grew in the light. A greater production of secondary roots in the dark was evident, both with and without the autolized yeast. The dry weight was somewhat greater in the light than in the dark. In the second period of growth, however, the difference between those roots grown in the light and those grown in the dark was very marked. Average length, secondary root production, and dry weight were distinctly greater in the light than in the dark. The difference was most marked in the set without autolized yeast. In the third period the roots in the dark in the modified Pfeffer's solution had ceased growth. In the fourth period those roots in the light in the Pfeffer's solution grew very little, and those in the dark in the solutions containing autolized yeast were discontinued because of the small amount of growth. In the fifth two-week period, six of the original eleven roots in the light in the modified Pfeffer's solution containing 80 ppm of yeast were still growing, and eight of the original ten roots in the light in the Pfeffer's solution containing 400 ppm of yeast were still growing. The number of roots decreased as continued transfers were made, due to contaminations or to the roots ceasing growth. Only two roots were left in the eighth period, one in the Pfeffer's solution

plus 80 ppm of yeast and one in the Pfeffer's solution plus 400 ppm of yeast. Both were transferred to solutions containing 400 ppm of autolized yeast and continued there. One of these became contaminated in the tenth period, and the other was accidentally discarded after growing nine days in the eleventh period, a total growth of 149 days under excised conditions. The individual records of these two roots are shown in table II. Figs. 1 and 2 represent graphically the average gain in length of the root tips grown under light and dark conditions in the Pfeffer's solution containing glucose, and in the same solution to which 400 ppm of autolized yeast were added.

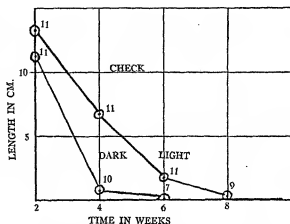


FIG. 1.—Average gain in length of excised corn root tips grown in modified Pfeffer's solution plus 2 per cent glucose in dark and in diffuse light; transfers of meristematic tissue made at two-week intervals; numbers on curves are number of root tips at each period.

In a second experiment (no. 21), begun December 11, 1922, root tips were grown in diffuse light both with and without auto-

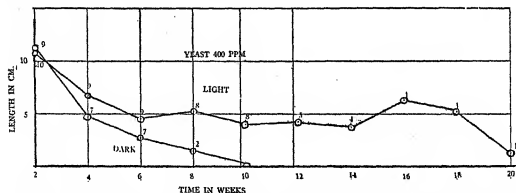


FIG. 2.—Average gain in length of excised root tips of corn grown in modified Pfeffer's solution plus 2 per cent glucose and 400 ppm of autolized yeast in dark and in diffused light; transfers of meristematic tissue made at two-week intervals; numbers on curves are number of root tips at each period.

lized yeast. Again the beneficial effect of the light was evident. Instead of ceasing growth in the third period, six of the ten roots

grew in diffuse light through six periods of transfer in the Pfeffer's solution containing 2 per cent glucose. All but one of the ten

TABLE I

GAINS IN LENGTH, SECONDARY ROOT PRODUCTION, AND DRY WEIGHT WHEN GROWN IN DARK OR IN DIFFUSE LIGHT IN MODIFIED PFEFFER'S SOLUTION PLUS 2 PER CENT GLUCOSE WITH OR WITHOUT AUTOLIZED YEAST; TRANSFERS OF MERISTEMATIC TISSUE MADE TO FRESH SOLUTIONS AT TWO-WEEK INTERVALS

Addition to Pfeffer's solution plus 2 per cent glucose	Condition	Period	No. roots	Average original length (cm.)	Average gain in length (cm.)	Average no. secondary roots	Dry weight per 10 roots
None.....	Light	1: April 2-16	11	1.8	13.1	48	0.1118
None.....	Dark		11	1.7	11.4	87	0.0838
80 ppm yeast...	Light		11	1.6	11.3	47	0.1094
	Dark		12	1.7	10.9	84	0.0913
400 ppm yeast...	Light		10	1.7	10.9	51	0.1134
	Dark		9	1.4	11.1	72	0.1035
None.....	Light	2: April 16-30	11	2.1	6.9	19.0	0.0163
None.....	Dark		10	1.9	0.8	5.9	0.0030
80 ppm yeast...	Light		9	1.6	8.7	33.0	0.0173
	Dark		9	2.0	3.8	7.0	0.0050
400 ppm yeast...	Light		9	2.0	6.6	22.0	0.0129
	Dark		7	1.8	4.8	16.0	0.0060
None.....	Light	3: April 30-May 14	11	1.8	1.9	5.5	0.0073
	Dark		7	1.3	0	0.1	0.0020
80 ppm yeast...	Light		9	2.0	4.1	7.7	0.0063
	Dark		9	1.7	0.8	0.5	0.0033
400 ppm yeast...	Light		9	2.1	4.5	8.7	0.0062
	Dark		7	1.8	2.9	1.7	0.0020
None.....	Light	4: May 14-30	9	1.2	0.3	1.3	0.0042
	Dark						
80 ppm yeast...	Light		7	1.6	3.5	5.0	0.0034
	Dark		4	1.7	0.1	0.0
400 ppm yeast...	Light		8	1.8	5.4	11.5	0.0082
	Dark		2	1.8	1.5	0
80 ppm yeast...	Light	5: May 30-June 13	6	1.6	1.5	3.5	0.0010
400 ppm yeast...	Light		8	1.7	4.0	12.1	0.0035
80 ppm yeast...	Light	6: June 13-27	5	1.3	2.7	1.8	0.0040
400 ppm yeast...	Light		5	1.5	4.2	9.4	0.0090
80 ppm yeast...	Light	7: June 27-July 11	3	1.6	2.9	4.6	0.0030
400 ppm yeast...	Light		4	1.5	3.8	5.5	0.0170
80 ppm yeast...	Light	8: July 11-25	2	1.7	2.1	3.5
400 ppm yeast...	Light		1	1.7	6.5	15.0
80 ppm yeast...	Light	9: July 25-August 9	1	1.6	6.5	4
400 ppm yeast...	Light		1	1.5	5.3	9
80 ppm yeast...	Light	10: Aug. 9-23	1	1.8	5.1	11
400 ppm yeast...	Light		1	1.3	1.2	1

ceased growth in the sixth period. This single root, however, was grown for ten periods, when the experiment was discontinued. In

diffuse light eight of the thirty-seven roots were still growing in the same solution plus 400 ppm of autolized yeast after ten periods of transfer.

In a third experiment (no. 23), begun December 17, 1922, the modified Pfeffer's solution plus 2 per cent glucose was used. Grains of corn with the root attached were placed in one series of flasks, a single grain to a flask. Part of the flasks were exposed to diffuse light and part placed in the dark. Root tips were severed at the same time from other grains and transferred to flasks, part of which were placed in the light and part in the dark. After two weeks growth the tips were severed and transferred to fresh flasks. At

TABLE II

GAINS IN LENGTH AND SECONDARY ROOT PRODUCTION FOR TEN PERIODS OF TWO OLDEST ROOTS IN EXPERIMENT-13; NO. 57 CONTAMINATED IN TENTH PERIOD; NO. 34 ACCIDENTALLY DISCARDED AFTER GROWING NINE DAYS IN ELEVENTH PERIOD

		Period										Total
		1	2	3	4	5	6	7	8	9	10	
Root No. 57	Gain in length (cm.)	14.0	6.8	4.5	6.6	6.3	8.6	8.0	6.5	5.3	1.2	67.8
	Secondary roots....	73	18	4	7	16	20	12	15	9	1	175
Root No. 34	Gain in length (cm.)	10.1	8.2	3.7	4.1	4.4	6.5	7.0	4.1	6.5	5.1	59.7
	Secondary roots....	9	32	7	1	4	9	14	7	4	11	98

two-week intervals further transfers were made, until growth ceased. There was thus one set of root tips in the light and one in the dark which grew for the first two weeks attached to a corn grain, and a second series of root tips which grew detached from the grain from the beginning of the experiment. The results are given in table III.

The beneficial effect of the light was less marked in this experiment than in the other two. During the first period both those roots attached to the grains and those severed grew much better in the dark than in the light. In the second and third periods little difference was evident. After the third period, however, a distinctly beneficial effect of the light was evident, both in the amount of growth and in the number of roots which survived. In the case of those root tips grown in the excised condition from the beginning

of the experiment, six of the ten in the light were still growing, while none in the dark were growing after four transfers. For those roots which grew attached to a grain for the first two weeks, the beneficial effect of the light was less noticeable.

This experiment also shows another fact of interest. Those roots which grew attached to a grain during the first two weeks

TABLE III

GAINS IN LENGTH AND SECONDARY ROOT PRODUCTION WHEN GROWN UNDER EXCISED CONDITIONS FROM BEGINNING, AND WHEN GROWN FOR FIRST TWO WEEKS ATTACHED TO GRAIN, IN DARK AND IN DIFFUSE LIGHT

PERIOD	CONDITION	DETACHED FROM START			ATTACHED DURING FIRST PERIOD		
		No. roots	Average gain per root (cm.)	Average no. secondary roots	No. roots	Average gain per root (cm.)	Average no. secondary roots
1.....	{Dark	16	10.25	50.0	13	26.7	108.0
	{Light	15	5.6	7.7	8	13.3	72.0
2.....	{Dark	11	2.2	9.0	10	6.2	30.6
	{Light	13	3.65	7.8	6	4.1	3.0
3.....	{Dark	11	0.38	0.9	10	1.46	4.8
	{Light	12	1.6	3.1	6	2.1	3.5
4.....	{Dark	1	1.6	0	9	1.4	1.0
	{Light	10	2.4	4.4	5	2.4	3.2
5.....	{Dark	1	1.6	0	2	3.2	1.5
	{Light	8	2.2	4.2	5	2.4	2.6
6.....	{Dark	2	0.6	0
	{Light	7	1.7	2.5	4	1.4	1.0
7.....	{Dark	1*	5.8	11
	{Light	6	2.1	2.3	1	2.4	0
8.....	{Dark	1†	0.7	3.0
	{Light

* Period was four weeks.

† Period was three weeks.

made a much greater growth in the second period than when they grew under excised conditions from the start. This beneficial effect is very evident in the third and fourth periods in the number of roots which survived, and is also evident in the fifth and sixth periods. In fact, one root tip grew a total of 133 days in the dark, 119 days under excised conditions. This is the longest period in which an excised corn root tip has been grown in the dark in the modified Pfeffer's solution.

Discussion

While diffuse light has a beneficial effect upon the growth of excised root tips of corn under the conditions of the experiments, the effect is not great enough to permit continued growth. In the later periods the roots become thin and threadlike, the elaboration of dry matter is slight, and the roots cease growth. The cause of the beneficial effect of the light is problematical. Does it aid in the elaboration of some accessory food substance, assist in the utilization of some material already present in the solution, or reduce the injurious action of some factor or factors which slowly cause the root tips to cease growth? The determination of the reason for the cessation of growth of the root tips will probably explain also the beneficial effect of the diffuse light. It is not due to the production of chlorophyll in the roots, although SIEBERT (5) has reported the development of chlorophyll in the roots of several plants in the light.

The beneficial effect on growth in later periods of permitting a root tip to remain attached to the grain during the first two weeks might be interpreted as substantiating the hypothesis that an accessory food substance is secured from the grain. After the root tip is separated from the grain it would depend upon the quantity of accessory material contained in it, and therefore the growth in the second period of those roots attached to the grain during the first period is much like the first period growth of the root tips excised from the start, and in the third period like that of the second period growth of the roots excised from the start. On the other hand, we might also assume that the stoppage of growth is due to the cumulative effect of some injurious factor, which is reduced because of the greater proportion of tissue present in the case of those roots which grew attached to the grain during the first period.

Summary

1. Diffuse light was favorable to the continued growth of root tips in a modified Pfeffer's solution containing 2 per cent glucose, or the same solution to which 80 ppm or 400 ppm of autolized yeast were added.
2. Anthocyan developed in some of the excised corn roots in the light, but in none in the dark.

3. The anthocyan pigment was present in the root cap, at points where secondary roots were to emerge or had emerged, and at the base of the root, but not in the meristematic region.

4. The longest period a root tip was grown was 149 days, through ten periods of transfer, in diffuse light in the modified Pfeffer's solution containing 2 per cent glucose and 400 ppm of autolized yeast.

5. Roots which remained attached to the grain during the first two weeks grew for a longer time than those grown under excised conditions from the start. One such root was grown in the dark for 133 days, 119 in excised condition.

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DEPARTMENT OF BOTANY
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SECONDARY STELAR STRUCTURES OF YUCCA
CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 327
GRACE BARKLEY
(WITH NINE FIGURES)

Yucca belongs to a group of monocotyledons peculiar in having a cambial ring producing secondary tissues which increase the size of the stem as do the secondary tissues of dicotyledons and gymnosperms. These secondary tissues are very characteristic of this group, since they differ in important features from those of gymnosperms and dicotyledons.

DEBARY¹ called attention to the secondary growth in the arborescent stems of *Dracaena*, *Yucca*, *Aloe*, and others, and described the group in general. He stated that the initial layer of cambium consisted of and remained a simple layer of cells, and that it must be regarded as belonging to the plerome cylinder. He also described the secondary tissues of *Dracaena*. SCHOUTE² stated in regard to this group that at first the cambial layer is regenerated at the expense of the cortical layer external to it, and later a definite initial layer is differentiated. He made some measurements of the thickness of the growth from the cambium, and tabulated the measurements of the primary and secondary cylinders of several of these stems, doing more work on *Dracaena*, but also including *Cordyline rubra* and *Yucca filamentosa*. CHAMBERLAIN³ announced the discovery of growth rings in the secondary tissues of *Aloe ferox*. He also described the primary and secondary bundles of this stem.

Most of the arborescent monocotyledons, like the herbaceous forms, do not have secondary growth. The diameter of their stems is determined by growth in the primary tissues and by the differentiation of these primary tissues, after which no further change

¹ DEBARY, A., Comparative anatomy of the Phanerogams. 1884.

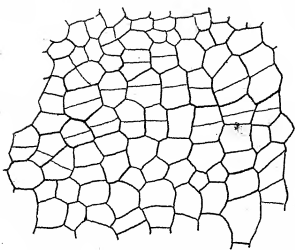
² SCHOUTE, J. C., Die Stammesbildung der Monocotylen. Flora 92:32-48. 1903.

³ CHAMBERLAIN, C. J., Growth rings in a Monocotyl. BOT. GAZ. 72:293-304. 1921.

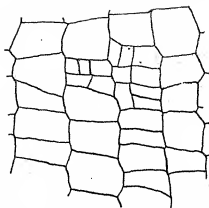
takes place. Like other monocots, *Yucca aloifolia* has a primary cylinder in which the bundles are scattered through the parenchyma. This cylinder is surrounded by the cylinder of secondary tissues composed of bundles scattered through parenchyma. The secondary cylinder is hard, and in the stem studied showed growth rings which, to the naked eye, resembled the growth rings of woody dicotyledons. This cylinder is surrounded by the primary cortex, which is inclosed by the secondary cortex.

The cambium ring first makes its appearance 1.5 cm. from the apex of the stem, originating in the pericycle. Several rows of cells begin to divide (fig. 1), making the initial cambium a zone of meristematic tissue. The young cambial cells have the radial diameter about one-third the other two dimensions, which are about equal. New tissue is produced on the inner side and becomes several cells thick before differentiation begins. The tissue produced by the cambium becomes differentiated into secondary bundles and parenchyma. A cell in one of the radial series from the cambium begins to divide radially and tangentially at first (fig. 2), and then in all directions, until numerous cells are formed. One or two adjacent cells from adjacent series may begin division at the same time and combine in forming a bundle, so that a bundle may be formed from one or two mother cells from one or two radial rows.

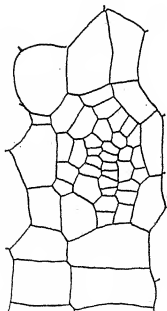
In the stem studied there were very distinct growth rings. These were formed by an increase in the number of vascular bundles, which are more numerous there than in the region between the rings (fig. 7). Generally there are three or four rows of parenchyma cells between the bundles of the same row in the rings, and about twice as many cells between the rows of bundles. The bundles are further separated in the region between the rings, and they are also further separated in a radial direction, the rows of bundles being about twice as far apart as in the rings. The bundles between the rings are a little larger than those in the more crowded region of the ring. The parenchyma becomes lignified in the growth rings, reaching a greater lignification in the older rings, and remains unligified in the region between the rings. The secondary bundles are soon differentiated into xylem and phloem. The phloem lies toward the periphery of the stem and is generally on the outside of



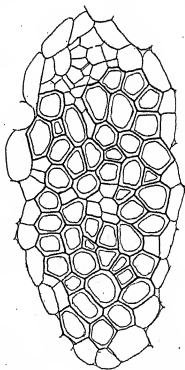
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FIGS. 1-4.—Fig. 1, initial cambium in several rows of cells; fig. 2, young bundle beginning with several mother cells; fig. 3, young bundle which originated in one radial row; fig. 4, usual secondary bundle.

the bundle. In some bundles the xylem almost surrounds the phloem, and sometimes a completely amphivasal condition is reached (fig. 5). This takes place where the bundles are more crowded.

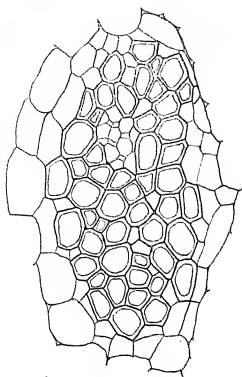
The xylem is composed of tracheids which have small, narrow, oblique bordered pits arranged in one or two rows on their radial and tangential walls, and a few thin walled parenchyma cells. The phloem is very small, and, in transverse section, shows six to eight sieve tubes with companion cells.

The secondary parenchyma is composed of cells usually longer than wide, with large intercellular spaces. All the cells have numerous round pits in their walls (fig. 6). The cells adjacent to the bundles have walls more thickened than the other cells, and this makes the pits more plainly visible in these regions.

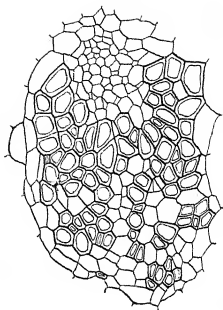
Sections of wounds were studied and the secondary growth noted here also. Two cambiums are produced in these wounds parallel to the face of the wound, a cork cambium and an inner cambium. The tissue produced on the inner side of the inner cambium is differentiated into bundles and parenchyma, as is that produced by the cambium of the stem. Considerable callus was built on the face of the wound before the cambium formed, and this is formed on the inner side of the callus near the bundles in the old tissue. These wound bundles are generally like the secondaries of the stem, with about the same amount of phloem and xylem. The phloem is always toward the cambium, and this makes the new bundle take various positions relative to the old bundles.

In some places the new bundles are suberized, and the lumen of the cells becomes filled as soon as the cells become differentiated. A sheath of parenchyma is also formed about these bundles, which becomes suberized. The intervening parenchyma remains unmodified. In most places only the outer part of the new cortex is suberized.

A peculiar modification of the new bundle was found in a place where they were formed under the influence of two wounds. These wounds were perpendicular to each other, and the end of one was 0.25 inch from the face of the other. Two rows of new bundles were formed parallel to the face of one of the wounds, but the



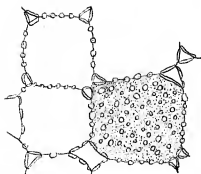
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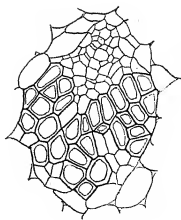
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FIGS. 5-9.—Fig. 5, amphivasal secondary bundle; fig. 6, radial section of parenchyma showing pits in walls; fig. 7, diagram showing distribution of secondary bundles in growth rings; fig. 8, larger primary bundle; fig. 9, smaller primary bundle.

phloem, instead of being on the side of the bundle toward the cambium that formed the bundle, was toward the other wound.

There are two kinds of primary bundles, one being larger than the other. The larger bundle, in transverse section, shows a small amount of phloem, about 20-30 sieve tubes and companion cells (fig. 8), located on the outer side of the bundle. The xylem of this bundle is composed of a few pitted vessels near the phloem, many tracheids with narrow oblique bordered pits, spiral vessels, and thin walled parenchyma. The smaller bundle, in similar section, shows less phloem, having about 10-20 sieve tubes and companion cells (fig. 9). The xylem has no pitted vessels and no protoxylem, and is composed of only the tracheids and parenchyma similar to those of the larger bundle.

The larger primary bundles are leaf traces, and they remain unchanged when they enter the stem, except that they lose the sheath. They have a cambium in the leaf base, which may be seen in the stem as far as 1.5 cm. from the end.

The leaf traces enter the primary cylinder and pass downward at an angle of about 25° toward the center of the stem, where they curve obliquely around the center and then pass downward on the opposite side, parallel to the axis of the stem. After secondary tissue is formed they pass through it horizontally until the primary cylinder is reached.

The material for this study was obtained from plants growing out of doors in the Gulf States, and from one of the greenhouses of the University of Chicago. The plant used in the study of the growth rings came from the greenhouse. It was about seven years old and was about 12 feet high, but it had been topped once. The main stem was 4 feet in length from the ground to the branches. It was 5 inches in diameter, with a 2 inch primary cylinder. The larger stems of the material from the Gulf States also showed growth rings.

It would be interesting to know to what extent growth rings are present in *Yucca* stems in their native habitat.

Summary

1. The cambium originates in the pericycle about 1.5 cm. from the end of the stem, and the initial cambium is a zone of meristematic tissue.

2. The secondary bundle originates from one or two cells in one or two radial series. They are usually collateral, but in some bundles the amphivasal condition is reached.

3. There were growth rings in the stems studied, formed by an increase in the number of bundles in alternating regions together with lignification of the parenchyma in these regions.

4. In the wound tissue a cambium is formed which produces a secondary tissue similar to the secondary tissue of the stem, except that some of the bundles are suberized, and these are surrounded by a sheath which is also suberized.

5. The leaf traces pass down the primary cylinder at an angle of about 25° , curve around the center of the cylinder, and continue on the opposite side parallel to the axis of the stem.

I am indebted to Professor CHARLES J. CHAMBERLAIN for suggestions and direction in this study.

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GROWTH-INHIBITING AND GROWTH-STIMULATING SUBSTANCES

W. F. GERICKE

When pots containing fertile soils are seeded to wheat or barley for continuous seasons, and successive short period crops of four to six weeks' growth are removed from such soils, it is found that the first crop is usually the largest, the immediately succeeding crops are much smaller, but some of the latter crops may be larger than some of the preceding ones. Thus instead of soils becoming progressively poorer by continuous cropping due to progressive diminution of its supply of plant food, they may become better at certain times than they were before, through certain processes and agencies concerned in the growth of plants. The results obtained with soils (7) led the writer to conduct experiments with culture solutions, and to employ a method that would provide a number of successively grown crops under the same climatic complex.

As the method required that the same nutrient solution be used repeatedly, large glass bottles of 6 liter capacity were used as the culture containers. These were filled with a nutrient solution of two atmospheres osmotic value, which was composed of equal partial molar concentrations of KH_2PO_4 , $\text{Ca}(\text{NO}_3)_2$, and MgSO_4 . The experiment was begun by planting a set of these containers with wheat seedlings. The plants were allowed to grow six weeks. They were then removed, and newly germinated seedlings, 4-6 cm. high, were placed in this set of containers. At the same time another set of containers having the original nutrient solution were likewise planted with wheat seedlings. The first set of containers, therefore, grew its second crop at the same time that the second set grew its first crop. Thus any number of crops grown successively and under the same climatic environment can be obtained by introducing a new set of culture containers into a series when the other sets thereof are replanted for progressive numbers of crops.

No addition of salts or renewal of nutrient solution was made during the course of the investigation, except to add distilled water

to the containers to replace loss by transpiration. At frequent intervals small quantities of FeSO_4 were added to the cultures to meet the demands of the plants for iron. Qualitative tests made at intervals during the investigation always showed a sufficient supply of nutritive elements in the culture solution. At the end of the experiment, the set of bottles which contained the nutrient solution that had supported seven crops still contained sufficient supply of salts to have supported several more crops. Some of the results obtained from this investigation are given in table I.

TABLE I

DRY WEIGHT IN GM. OF SUCCESSIVE CROPS GROWN CONTEMPORANEOUSLY AND UNDER SAME CLIMATIC CONDITIONS; AVERAGE OF FOUR CULTURES PER SET

Series no.	First	Second	Third	Fourth	Fifth	Sixth	Seventh
7.....	6.0±.07*	3.5±.27	4.4±.01	5.5±.06	4.9±.20	4.2±.20	4.6±.10
6.....	4.8±.23	3.4±.10	4.7±.10	3.8±.13	4.6±.13	4.4±.27
5.....	3.4±.03	2.8±.02	3.4±.13	3.0±.10	2.6±.02
4.....	2.0±.13	2.7±.10	1.7±.10	2.8±.14
3.....	3.3±.13	2.5±.02	3.3±.02
2.....	1.6±.00	1.5±.01
1.....	1.7±.10

* Two cultures.

The "first crop" column gives the dry matter produced from wheat cultures grown in seven different series of containers filled with the original nutrient solution. They are crops grown under different climatic conditions. These first crop cultures followed one after the other in sequence of time, and there was no intermission between the harvesting of one crop and the replanting of new media with new seedlings. The growth period of each series was six weeks, except that of 1 and 2, which was one month.

All crops of any series were grown under the same climatic conditions, the same length of time, and in the same kind of nutrient solution, except in respect to such changes as have occurred incident to, or as the result of, the crops it had grown. Referring now to the crops of series 7, it is to be noted that the first crop was the largest and the second was the smallest of the series. All succeeding crops were markedly larger than the second. The depression in yield of the second crop must be accounted for in the properties of the

nutrient solution derived from, or being, the effects of the first crop. That is, either toxic substances have been produced by the first crop, or the decreased yield of the second crop was due to a diminution in the supply of plant food removed in the first crop. Due to the fact that succeeding crops did not show corresponding decreases in yield as a result of obvious diminution of nutritive elements in the culture media, however, it follows that the explanation must be sought in the first premise.

The results in series 6, 5, and 3 are similar to those of series 7, that is, a depression of yield follows the first crop and the third crop shows marked improvement over the second. Series 4 gave results somewhat different from the others, in that the second crop was the largest and the third crop was the smallest; but, similar to the results in all the other series, increased yield ultimately followed a low yield.

Comparisons between the yield of the second crop and the third crop of series 7, 6, 5, and 3, and between the third crop and the fourth crop of series 4, show that the average of the yields of the succeeding crops in question is approximately 36 per cent greater than the immediately preceding one. It appears from these data that the harmful effects due to the growth of the first crop, as evidenced in the depression of yield in the second (or third in series 4), did not exist for the third crop. The average yield of the third crop for all comparable series was only 8 per cent less than that of the first. In some cases, the yield of a succeeding crop was equal to that of the first. While the media in which the plants of the second crop were rooted produced markedly inferior plants, nevertheless the reaction and effect of the second crop on these solutions, which became the growth media for the third crop, were decidedly beneficial. That is, it seems that a growth stimulating substance was produced by the plants in the second crop, which benefited the seedlings placed in these solutions and accounted for the improvement in yield of the third crop. On the other hand, it may be argued that the harmful properties due to the first crop had been made innocuous or nearly so by the second crop, and therefore the improvement in yield of the third crop was not due necessarily to the production of growth stimulating substances by the second.

That growth inhibiting substances may be produced by plants has been suggested by some of the early botanists (5) as an explanation for certain ecological distributions of plants. Later, when critical study was given to problems of soil fertility and crop rotation, this matter was given more serious consideration. Through extensive investigations in this field, LIVINGSTON, BRITTON, and REID (9), LIVINGSTON (8), SCHREINER and REED (11), SCHREINER and SHOREY (10), and others came to the conclusion that soils may be infertile because of the presence of some substance or substances produced by the plants or resulting from the decay of vegetation that may be inimical to crop production. Investigations on the effect of one crop on another by BEDFORD and PICKERING (1) show that one plant may affect other plants deleteriously. The conclusions arrived at by these investigators are in part as follows:

(1) Every growing crop results in the formation of a substance which is toxic to the growth of other plants and still more to itself.

(2) By oxidation, these toxins lose their toxic properties and enhance the fertility of the soil. The plants previously poisoned eventually outstrip those which had not been subjected to the poisoning, except in cases where the toxic effect has been sufficient to produce stunting.

(3) The toxic effect must necessarily vary considerably with the different conditions, both as to the nature of the soil, the plant affected, and the vigor of growth of the plant producing the toxin.

(4) There is no reason for assuming the excretion of any toxic matter from a plant, the debris from the growing roots is probably sufficient to account for the formation of the toxin.

Field experiments conducted by FLETCHER (6) at Gizeh, Egypt, also bear out the conclusions relative to the deleterious effect of one crop upon another. These experiments were especially designed to test these points, as FLETCHER had conducted certain water culture experiments which led him to the conclusion that plants excrete toxic substances.

That organic plant constituents added to the culture media may have beneficial effects on vegetation has been shown by several investigators. Among these, the workers in the Department of Agriculture already referred to have shown that these beneficial effects may be accounted for by (1) rendering toxic substances innocuous, (2) utilization as nutrients. That toxic material upon

oxidation may prove decidedly beneficial to plants is the conclusion arrived at by BEDFORD and PICKERING, in the investigations already referred to. The investigations by BOTTOMLEY (2, 3, 4) with growth producing substances (auximones) derived from bacterized peat show that not only may the products of organic growth be beneficial to plants, but they may be essential and indispensable. Furthermore, as minute quantities of this material appear to suffice, it appears that the rôle this material plays in plant nutrition must be considered in a different light from that ordinarily accorded to the essential mineral elements.

The growth of plants is not only a complex of many processes, but is in a sense the result of the succession of processes. Some of these processes are dependent on certain salts which supply necessary inorganic elements to plants. In order that the plant may have these elements when needed, it is essential that there be sufficient supply in the culture media to meet the demands of the plant. The question may be asked in this connection, Are subsequent stages of growth dependent only on certain inorganic elements in media in the sense that these would supply the salt requirement for any particular growth phase? May not small amounts of substances of unknown composition arising from previous or contemporaneous growth processes of plants be essential for subsequent phases of growth in the same sense as are mineral elements in the culture media? Apparently the final answer to this question is not yet at hand.

The fact that plants may change the reaction of a culture media from a less to a more favorable condition is at least suggestive of an affirmative potentiality of this query. Furthermore, experimental data show that a constantly maintained supply of nutrients in culture media is not as favorable to the growth (entire life cycle) of cereal plants as is a changing one. This may indicate that any subsequent stage of growth may be beneficially affected by secretions from previous growth phases. That is, plants may be similar to animals in that they have a physiology which is subject to growth inhibiting, growth stimulating, and sensitizing agencies produced by the organism itself.

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INHERITED DEFICIENCY IN CARBOHYDRATE METABOLISM IN MAIZE¹

WILLIAM H. EYSTER

(WITH THREE FIGURES)

When commercial varieties of maize are inbred, a relatively large percentage of them give progenies that segregate one or more types of chlorotic seedlings. The chlorotic conditions are due to genetic factors which in some way influence the development of the chloroplastid pigments. Albino plants are produced when the chloroplastid pigments are entirely lacking, although in some types a small amount of the pigments is developed, especially at the leaf tips and along the veins, so that they become cream colored. The life of such plants is limited to the food reserve in the endosperm, as they are unable to synthesize carbohydrates. A few types of albinos have been found, which, under the most favorable conditions, develop the chloroplastid pigments sufficiently to enable them to grow to a mature, although very weak plant, as illustrated by *virescent white*.²

Yellow seedlings are produced when there is a partial or complete development of carotin and xanthophyl, with only a slight development or complete absence of one or both of the chlorophyls (chlorophyl *a* and chlorophyl *b*). When the chlorophyls are entirely lacking, the yellow seedlings vary in color from cream-yellow to bright yellow, according to the amount of yellow pigment developed. The deep yellow types usually have small amounts of one or both of the chlorophyls, in addition to carotin and xanthophyl. Genetic types of maize have been found also which range from deep yellow, through various shades of yellow-golden, green, and olive green, to full green, according to the relative amounts of the different chloroplastid pigments produced.

¹ The investigation reported in this paper was made in connection with genetic studies of maize in the Department of Field Crops, Missouri Agricultural Experiment Station.

² LINDSTROM, E. W., Chlorophyl inheritance in maize. Cornell Univ. Agric. Exp. Sta. Mem. 13: 1-68. 1918.

Preliminary studies³ of a quantitative nature indicate that yellow seedlings which develop carotin and xanthophyl, but lack both chlorophyl *a* and chlorophyl *b*, are not able to synthesize carbohydrates. Types were found which contained (1) carotin, xanthophyl, and chlorophyl *a*, but lacked chlorophyl *b*; (2) carotin, xanthophyl, and chlorophyl *b*, but lacked chlorophyl *a*; and (3) carotin, xanthophyl, chlorophyl *a*, and chlorophyl *b* in varying amounts. So far as studies have been made, plants which lack either chlorophyl *a* or chlorophyl *b* are unable to synthesize sufficient carbohydrates to grow to maturity, but it was interesting to find that the two chlorophyls appear to be of unequal importance, as indicated by the length of time the different types were able to live after the food reserve of the endosperm was exhausted. The time between the complete digestion of the endosperm and death may be said, in general, to increase with the amount of the chlorophyls that develop in the chloroplastids, until they are sufficiently developed to enable the plant to synthesize the carbohydrates necessary to grow to a mature though very weak plant. Vigor increases as chlorophyl content approaches that of a normal green plant, although it is not necessary to have a full complement of the green pigments to produce a plant that makes an apparently normal growth, as is well illustrated by *golden* (LINDSTROM, 1918).

In all the genetic types mentioned, the inherited characters are concerned with the development of the chloroplastid pigments. All of them are able to utilize the carbohydrates stored in the endosperm, and live normally while they last. In the types which develop the chloroplastid pigments in sufficient amounts, life is prolonged according to the ability of the chloroplastids to synthesize carbohydrates.

In the summer of 1920 I found in cultures of maize a yellow seedling which differs from other chlorotic seedlings, in that it is unable to utilize the carbohydrates in the endosperm. Under field conditions this type of yellow seedling usually dies within a week after the appearance of the first leaf, while under more favorable

³ The writer is indebted to the Bussey Institution of Harvard University, and especially to Dr. E. M. East, for facilities for making quantitative studies of the chloroplastid pigments in various genetic types of maize.

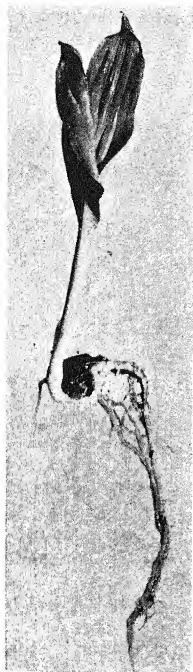


FIG. 1

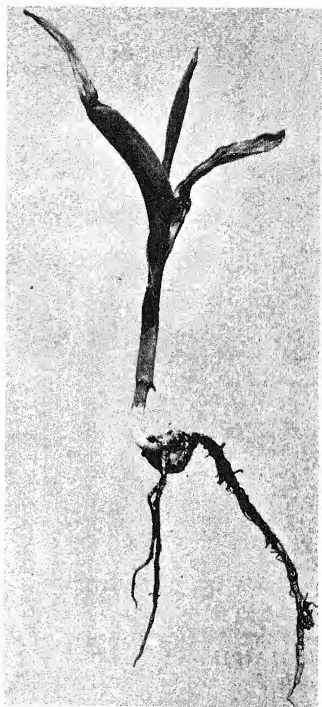


FIG. 2

FIGS. 1, 2.—Maize seedling showing evidences of glucostacty less than a day after appearance of first leaf; fig. 2, glucostactous maize seedling with first leaf badly wilted, and second and third leaves partly autolyzed; evidences of glucose on ends of first and second leaves.

conditions in the greenhouse it has been kept alive for a longer period. The plant is deficient in the chloroplastid pigments, as shown by its yellow color. That the plant's deficiency extends beyond the development of the chloroplastid pigments and the synthesis of carbohydrates is indicated by the early autolysis and death of the young leaves. Autolysis begins almost as soon as the leaves are unrolled. The tips of the young leaves appear water soaked, and this condition rapidly extends down toward the base of the leaf. Soon the leaves appear wilted, and exude a liquid which at first is clear, but soon changes to a light brown and finally a dark brown color. Plants in this condition were observed to attract flies, and, upon tasting, the liquid was found to be quite sweet. This brown, sweet tasting liquid was analyzed⁴ and found to be glucose with a trace of maltose. It was interesting to find that not even a trace of fructose was present.

The plant illustrated in fig. 1 was photographed less than a day after the appearance of the first leaf. It may be seen that the tips of the first and second leaves show evidences of autolysis. Fig. 2

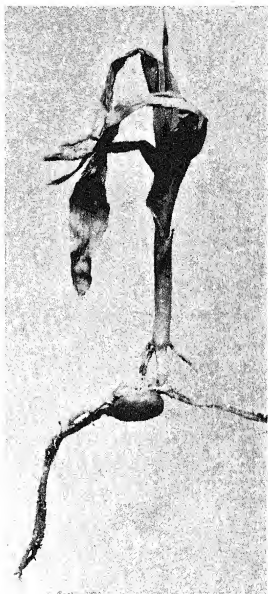


FIG. 3.—Glucostactous maize seedling illustrating condition typical of plants at end of about a week after appearance of first leaf.

⁴ The analysis was made by Dr. M. M. ELLIS, University of Missouri, by the osazone method.

shows the first leaf completely wilted, while the second and third leaves are partly autolyzed. There are evidences also of the brown liquid on the ends of the leaves. The plant shown in fig. 3 had all

TABLE I
F₂ PROGENIES FROM CROSSES EQUIVALENT TO
normal × *glucostactous*

PEDIGREE	TYPE OF SEEDLING	
	Normal	Glucostactous
1088- 2(X)	105	27
- 3(X)	101	31
- 4(X)	61	18
-10(X)	68	24
-13(X)	88	27
-14(X)	107	27
-15(X)	71	43
-18(X)	58	19
1089- 1(X)	74	24
- 3(X)	105	37
- 5(X)	78	25
- 7(X)	85	32
- 9(X)	67	27
1091- 7(X)	101	47
1093- 4(X)	65	32
1094-18(X)	98	35
1099-12(X)	61	26
1572- 5(X)	107	36
-11(X)	31	19
-12(X)	57	18
-25(X)	58	11
-32(X)	30	15
-39(X)	43	17
-41(X)	59	17
-46(X)	16	5
-47(X)	37	7
-50(X)	33	13
-52(X)	20	5
1586-13(X)	113	31
- 8(X)	141	44
-12(X)	135	48
340- 4(X)	123	46
675- 8(X)	83	37
675- 5(X)	234	75
674- 1(X)	55	25
673- 4(X)	192	77
673- 1(X)	252	99
674- 2(X)	18	4
5055- 3(X)	264	64
Total	3494	1214
Expected (3:1)	3531	1177
Deviation	37 ± 63.3	

of the leaves badly wilted in less than four days after the appearance of the first leaf. These plants have all the appearances of being affected by a wilt. Attempts were made to inoculate other plants, with negative results. The roots were found to be perfectly normal as compared with the roots of the green plants of the same progenies.

The death of the yellow seedlings just described is not due to a lack of carbohydrates, but rather to an inability to utilize carbohydrates that are available, and are even present in the tissues in soluble form. The presence of large quantities of glucose in the

TABLE II
F₂ PROGENIES OF CROSSES EQUIVALENT TO
normal × *glucostactous*

Pedigree	Progenies all green	Progenies segregated <i>normal</i> and <i>glucostactous</i>
1088.....	5	8
1089.....	0	6
1090.....	1	0
1091.....	5	1
1092.....	1	0
1093.....	1	1
1094.....	2	1
1099.....	0	1
1572.....	6	12
1586.....	0	4
Total.....	21	34
Expected (1:2).....	18	37
Deviation.....	+3	-3

tissues of the dying seedlings indicates that the carbohydrates stored in the endosperm are digested and absorbed, but are not utilized in the plant's vital processes. As a result, the glucose accumulates and finally is exuded from the tips of the leaves to form drops. The physiological condition in plants represented by these sugar-sweating maize seedlings may be called *glucostacty*.

This inability of the plant to utilize the sugars that are already absorbed is strikingly like the diabetic condition in animals. Whether the similarity is real, or only apparent, must be determined by further physiological study.

Glucostacty, which represents a profound disturbance in the carbohydrate metabolism, is inherited as a simple Mendelian

recessive character, as is shown by the data recorded in tables I and II. The symbol *G_l* will be used in referring to the gene responsible for this deficiency in carbohydrate metabolism. In table I are listed F_2 progenies with a total of 4708 plants, of which 3494 were green and 1214 were yellow and unable to utilize their absorbed sugars. This is a deviation of 37 ± 63.3 from the numbers expected for a 3:1 ratio. In table II are listed 55 F_3 progenies, of which 21 consisted of all normal green plants, and 34 segregated green and glucostactous seedlings as shown in table I. This is a deviation of about 3 plants from the 1:2 relation expected.

Summary

Of common occurrence are inherited chlorotic types of maize which are partially or wholly deficient in one or more of the chloroplastid pigments. The first source of carbohydrates for maize seedlings is the endosperm. When this supply is exhausted the plant dies, unless it is able to synthesize additional carbohydrates sufficient to maintain life. A genetic type of maize has been found and described that digests and absorbs the endosperm, but is unable to utilize the sugars thus obtained in the vital processes. This deficiency in carbohydrate metabolism has been called glucostacty, and is inherited as a simple Mendelian recessive character.

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TYLOSES IN MENISPERMUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 328

LYNETTE MYERS

(WITH PLATES XI, XII)

Tyloses were found as long ago as 1675, when MALPIGHI (8) found ingrowths in the spiral vessels of the oak which to him resembled the air tubes of insects. Since then many have investigated their distribution, origin, behavior, and function.

Probably all investigators have found tyloses in many woody angiosperms, some of which have more abundant formations than others. GERRY (4) made a report on many American woods; MOLISCH (9) examined several hundred vascular plants, and decided that tyloses are not formed in vascular cryptogams and gymnosperms. WILLIAMSON (15) and WEISS (14) found them in the fossil fern *Rachiopteris*. WEISS stated that it would be impossible for tyloses to be formed in gymnosperms, as these plants do not usually have parenchyma; but in 1908 CHRYSLER (2) investigated some gymnosperms and found tyloses in them.

For more than a century, tyloses were considered swellings of the inner walls of the tracheae. In 1845 SCHLEIDEN (11) observed that they enter the vessels from the parenchyma, but this was disputed by BOEHM (1) and UNGER (13). UNGER claimed tyloses to be bulges of the inner layer of the vessels, but BOEHM believed them to be formed by a collection of plasma between the lamellae of the vessel walls, the inner layer growing into the tylose. REES (10) proved these views wrong, including figures showing tyloses entering the vessels. Later investigators (3, 6, 8) agree that tyloses originate from adjacent parenchyma cells, entering tracheae through their pits.

Growth of tyloses has been observed by many investigators, and their division was noted as early as 1847 (12). DE BARY (3), describing growth changes, states that the protrusion, at first short, blunt, and cylindrical, expands into a round and sometimes volumi-

nous bladder containing protoplasm and usually a well defined nucleus, and finally separates from the cell which produced it by forming a wall at its point of entrance into the vessel.

Functions of tyloses have been observed as follows. (1) They close the ends of conducting tubes that have been injured, as shown by their extensive formation at ends of cuttings and beneath scars left from broken branches. In both situations their number and growth are so great that they become flattened from contact pressure (1, 5, 6, 13). (2) The sclerotic tyloses add to the mechanical strength of the heartwood; in winter, some tyloses are receptacles for starch (5, 6); when starch is present, tyloses may assist in conduction by increasing the surface of contact between the vessels and the parenchyma cells; by acting in the capacity of haustoria, they may increase the exudation pressure, excrete sugar, or withdraw certain sap constituents.

Material and methods

The material used for the study of *Menispermum* was collected from the forests adjacent to the Des Plaines River, Cook County, Illinois. Specimens varied from 1 m. to 8 mm. in diameter, and were taken at intervals of six inches from ground to tip, a distance of ten or twelve feet. These parts of the stem were fixed in a solution of formalin, alcohol, and acetic acid as soon as collected, the fixing solution being taken to the place of collection, that no change might take place in the material.

Some very good results came from sections of the fresh materials. Probably the best results were obtained from freehand sections stained with safranin and Grubler's light green, and cleared with clove oil, but paraffin sections were also used and they showed some details very clearly. Cellulose acetate was tried, but it does not soften this wood sufficiently to yield smooth paraffin sections.

Investigation

A preliminary study was made during the summer of 1920, and tyloses were found to be very abundant. The tracheae near the pith in most cases were filled with these growths (fig. 1), but vessels near the outer part of the stem did not contain any. These tyloses,

although abundant, seemed to be in a mature stage, as no new ones were present; neither was there any proof of division, although some had the appearance of having divided (fig. 10). It was quite evident that it would be necessary to collect material at different times of the year. This was done, the collections covering a period from July 1, 1921, to February 1, 1922.

The first new formations (figs. 2, 3) were found in material collected the second week of December. After this time the formations increased in number, some vessels being completely filled by February 1 (fig. 6). Sections showed tyloses entering the tracheae from adjoining parenchyma cells (figs. 2, 3, 9, 10). It is quite evident that a tylose is formed where the plain pit of a parenchyma cell comes in contact with a bordered pit of a trachea. This, however, would localize tyloses, as parenchyma pits formed in the spring are radial, while those formed in the fall are tangential (7).

Tyloses increased in size, their walls became very thick (fig. 4), and they were separated from the parent cells by cross walls. After a considerable increase in size, mitotic figures began to appear (fig. 11). These were found in different stages, including the telophase (fig. 12) where the wall is formed. More work should be done as to localization and mitotic figures.

Tyloses have many pits in their walls, material taken in January having tyloses in which they were abundant (figs. 7, 8). It is quite evident that intertylosing is a natural consequence where two tylose pits meet (figs. 5, 7). Many tyloses originate from other tyloses by means of these pits (figs. 7, 8).

Just outside the pith are several rows of cells which seem to present a peculiar lateral tylosing, but this has not been established. These cells have much the appearance of the anomalous secondary wood of *Lyginodendron*, and in places look much like phellogen. Their study is difficult because of a brown coloration which is due to an oxidase, not a pigment. This coloration is not continuous, being scattered promiscuously throughout the stem, but always confined to these cells and adjacent tissues.

Although *Menispermum* usually twines around a small tree or shrub, it may extend from one to another, and as these sway in the wind, such severe strains are given the dependent plant that it is

common to find the medullary rays so ruptured that the vascular bundles become more or less separated, giving the stem the appearance of a cable of separate strands. Nature seems to compensate for this injury by strengthening the stem with tyloses.

Summary

1. This paper reports the results of a study of tyloses in *Menispermum*, with reference to time of formation, structure, and behavior.

2. Sections showed no new formations until the second week of December, after which formations were rapid, some vessels being completely filled by February 1.

3. At this time the tyloses were filled with active protoplasm, and in most instances each tylose contained the nucleus which had passed into it, probably facilitating its growth.

4. Intertylosing was common where pits of different tyloses came in contact.

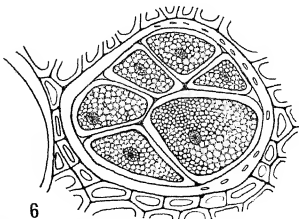
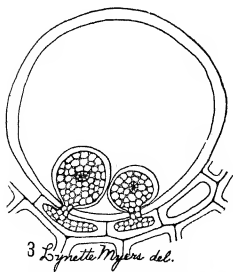
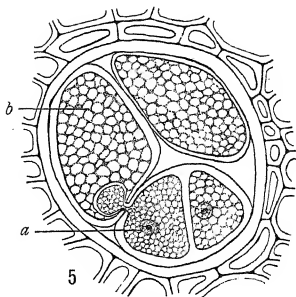
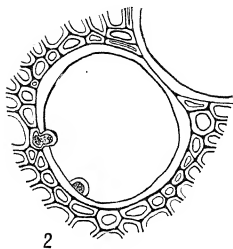
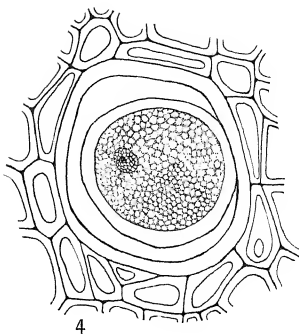
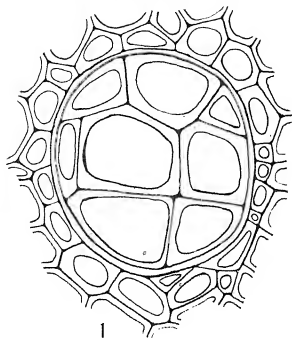
5. That tyloses divide was established by different stages of mitosis.

This study was suggested by Professor CHARLES J. CHAMBERLAIN, to whom sincere appreciation is due for criticism and suggestions.

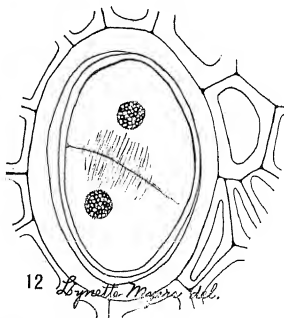
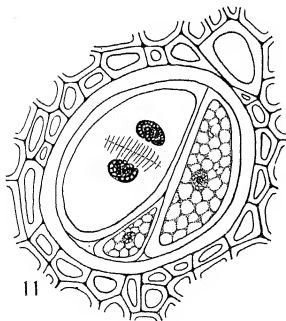
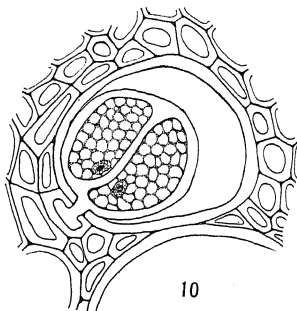
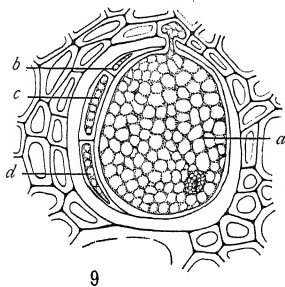
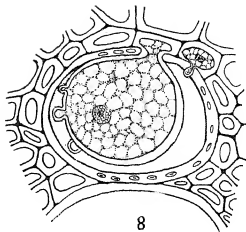
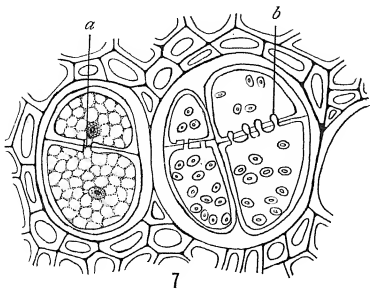
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► EXPLANATION OF PLATES XI, XII

FIG. 1.—Tylose situation typical of July or August, vessel crowded with tyloses showing no cell contents.

FIG. 2.—Young tylose with nucleus still in parenchyma cell.

FIG. 3.—Young tyloses containing nuclei.

FIG. 4.—Tylose as it appears first week of January; size greatly increased.

FIG. 5.—Tylose (a) forming tylose in an adjoining tylose (b).

FIG. 6.—Typical February 1 situation; tracheae filled with tyloses having active cell contents.

FIG. 7.—Tyloses having conspicuous pits; pit of adjoining tyloses meeting (a); intertylose formation (b).

FIG. 8.—Large tylose from which other tyloses are being formed.

FIG. 9.—Tylose (a) from which tyloses (b, c, d) may have originated.

FIG. 10.—Tylose having appearance of having divided.

FIG. 11.—Tylose dividing; mitotic figures near center.

FIG. 12.—Tylose division almost completed; wall being formed.

BRIEFER ARTICLES

DETACHMENT (ELIMINATION) OF CHROMOSOMES IN CYPRIPEDIUM ACAULE

(WITH TWO FIGURES)

In the fall of 1922, three plants of *C. acaule* were removed from the woods to pots in a cold frame. Early in 1923 they were transferred to the greenhouse. On April 3, half of an anther was examined from one plant. Almost all of the young pollen grains had only one nucleus. Some were in the metaphase, however, and among these six groups of ten V-shaped chromosomes were counted (fig. 1). One microcyte was

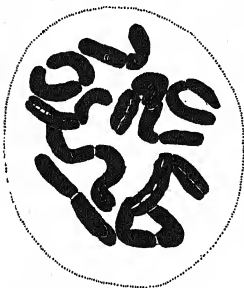


FIG. 1.—Metaphase of first division in pollen grain: ten V-shaped chromosomes show constriction at apex of V; fissure for longitudinal division obvious at this stage.

found, whose nucleus was about the size of a chromosome. This showed that detachment had occurred in at least one case; therefore more samples were examined. On the next day, most of the pollen grains had finished the division. The whole slide showed only nine cells with ten chromosomes, which, although they differed somewhat in length, were long and V-shaped in eight of the cells, and short and thick in one exceptional case. There were also eight cells with nine short and nearly straight chromosomes each (fig. 2). Four microcytes were seen, as well as five micronuclei in otherwise normal grains, but no special search was made for these.

Here we have evidence of at least four cases of detachment in the previous maturation divisions, probably at the first division. Another anther-half from the same plant showed most of the microspores with two nuclei. Twelve cells had ten long V-chromosomes, and one cell with nine short chromosomes was seen;

while five microcytes and one micronucleus were found without special search.

From the second plant (the third plant did not flower) a sample showed cells with ten long chromosomes, while no microspores with short

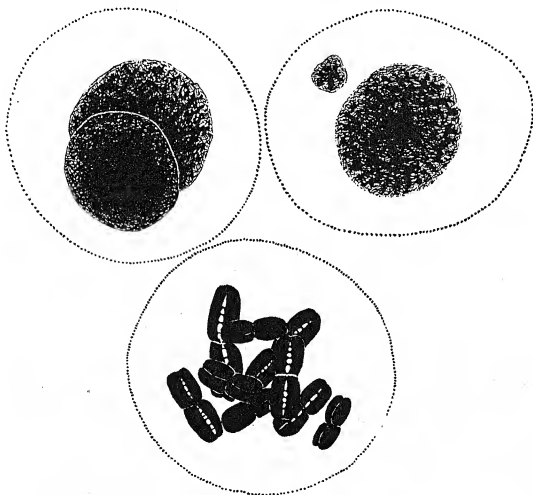


FIG. 2.—Three adjacent microspores: left-hand upper cell has completed nuclear division; in right-hand grain division has not begun, there is a micronucleus in cytoplasm, which arose probably from detached chromosome at preceding reduction division; lower pollen grain contains only nine chromosomes, which are comparatively short and straight.

(Both figures drawn with the camera, with a 2.5 mm. apochromatic water-immersion objective, and Wratten light-filter no. 56; preparations in iron-acetocarmine).

chromosomes were seen. (One microspore of double volume, and with a nucleus of double size, pointed to a case of non-reduction.) This flower bud, however, was examined too early to ascertain the presence of 9-chromosome microspores.

An obvious hypothesis is that the sudden change to warmth and the hastening of the flowering had produced these anomalies. It has not yet been shown, however, that they do not exist in the plants in the woods. The shortened state of the chromosomes, and the relative abundance of the 9-chromosome condition in the last cells to be found in the metaphase stage, may lead to the assumption that the microspores with one chromosome lacking were either longer in finishing their division than those with the complete group of ten, or perhaps did not complete their division at all (and so did not produce vegetative and generative nuclei), but aborted, as is the case with microspores with deficient chromosome numbers in the haploid *Datura*, and some other plants.—JOHN BELLING, *Carnegie Institution of Washington, Department of Genetics, Cold Spring Harbor, N.Y.*

GRINDING WOOD SAMPLES FOR ANALYSIS

(WITH ONE FIGURE)

The preparation of samples of woody tissues for the analysis of physiologically important changes in constituents in the past has been a rather difficult task, or has been accomplished only by expensive grinders which all too frequently require large samples for operation. It has been difficult to cut or grind with the ball mill, or other grinding equipment found on the market, such tissues as barberry roots or apple limbs into particles sufficiently small for easy extraction.

The writers adopted the idea of using a pencil sharpener for preparing these hard woody tissues for extraction, the Dexter pencil sharpener manufactured by the Automatic Pencil Sharpener Company of Chicago being used. This sharpener is provided with a strong frame and has good bearings which can be lubricated. There is provided also a convenient container for catching the ground sample. The machine can be operated by hand and costs little. Samples of twigs or split wood up to half an inch in diameter may be ground directly. There is no difficulty in recovering the sample quantitatively after grinding, and the machine can be cleaned quickly and easily.

The hardest dry oak or the softest green twigs may be ground, and each will come out in a finely comminuted condition suitable for extraction by alcohol or other solvent. For the analysis of apple twigs we have used the machine driven by a $\frac{1}{8}$ H.P. motor through a 1-10 reducing pulley. A sample of 100 gm. apple twigs can be ground in less than five

minutes. There is no heating of the cutters, even when grinding hard oak wood at the full capacity of the motor. It is best to press the material into the machine an inch at a time and then allow the motor to pick up speed to clean the cutters. It is possible to grind the material

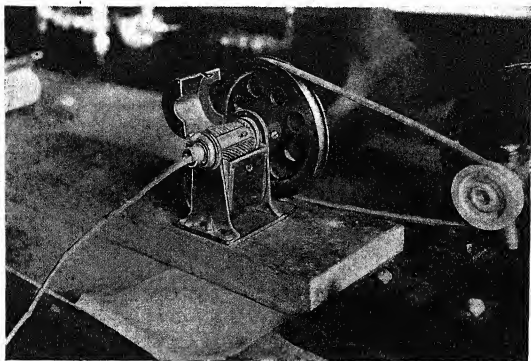


FIG. 1

faster in this manner than by feeding the sticks into the cutters by continuous pressure.—A. C. HILDRETH and R. B. HARVEY, *University Farm, St. Paul, Minn.*

ADVENTITIOUS SHOOTS ON HYPOCOTYL OF FLAX AND TOMATO

(WITH ONE FIGURE)

As a general rule, when the stem of a seedling dicotyledon is cut just above the level of the cotyledons, two branches are formed, one in the axil of each cotyledon. If the stem is cut below the cotyledons, however, the seedling in most plants dies. The result is different, however, in the case of flax.

During some experiments in May, 1920, with flax growing in pots in a greenhouse, as the seedlings were too numerous and as I did not wish to

disturb the roots of the others by pulling up those that were not wanted, I thought it would serve the purpose equally well to behead some of the seedlings with a pair of scissors. Instead of dying they remained perfectly green, however, and at length proceeded to form adventitious buds. The same result has been observed for three years in succession. From one to seven buds may arise on the hypocotyl, but usually only one (or sometimes two) grows out to form a leafy shoot, which may reach a

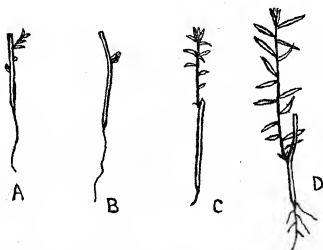


FIG. 1.—Adventitious buds on hypocotyl of flax: in *B* one bud is developing; in *A* two buds and one leafy shoot are seen; in *C* and *D* later stages of development are seen; *A*, *B*, *C*, natural size; *D*, half natural size.

length of 275 mm. These buds never originate from the callus which covers the cut end of the hypocotyl, but always at a considerable distance below it (fig. 1). The appearance of these adventitious buds is favored by the moist air of the greenhouse, their occurrence being much rarer when the seedlings are growing in the open.

The formation of similar buds has also been observed on tomato seedlings in the same greenhouse, but they are much rarer in their occurrence in this plant, and in all the seedlings examined they originated from the callus covering the cut surface of the hypocotyl.—J. ADAMS, *Central Experimental Farm, Ottawa, Canada.*

CURRENT LITERATURE

BOOK REVIEWS

Some notable European botanical textbooks

During the past two years there has been such a thorough revision of some standard European botanical texts, that they deserve at least the attention accorded to new editions. Among these, a prominent place should be given to WETTSTEIN'S *Handbuch der Systematischen Botanik*;¹ *Lehrbuch der Botanik*, by FITTING, JOST, SCHENCK, and KARSTEN;² *Das Botanische Praktikum*, by STRASBURGER;³ and to MASSART'S *Éléments de Biologie Générale et de Botanique*.⁴

WETTSTEIN'S book appeared in 1901, and the second edition, much enlarged and improved, came in 1911. It had been out of print for some time when the first volume of the third edition was published in 1923, followed by the second volume in 1924. The title would lead one to expect a treatise on taxonomy, but the whole field of modern morphology is presented, with emphasis on critical details of life histories, thus laying a foundation for a phylogenetic classification. The descriptions are accompanied by a wealth of illustrations seldom seen in a textbook. While many will not approve the position assigned to one group or another, all must admit that the assignments have been made after an exhaustive study of the literature, in addition to an extensive first hand knowledge of plants.

WETTSTEIN divides the whole plant kingdom into seven great lines, or phyla, between which phylogenetic relationship has not been proved. The content of some of these is so evident that no comment need be added. The phyla are as follows: I. Myxophyta; II. Schizophyta; III. Zygomycyta; IV. Phaeophyta; V. Rhodophyta (including Bangiae and Florideae); VI. Euthalophyta (including Chlorophyceae, Phycomycetes, Ascomycetes, Basidiomycetes, Fungi Imperfecti, and Lichens); VII. Cormophyta (including Bryophytes, Pteridophytes, Gymnosperms, and Angiosperms). The series of groups used throughout is phylum (Stamm), division (Abteilung), class, order,

¹ WETTSTEIN, R., *Handbuch der Systematischen Botanik*. 3d ed. pp. viii+1017. Pt. I, 1923; Pt. II, 1924. Leipzig & Vienna: Franz Deuticke.

² FITTING, H., JOST, L., SCHENCK, H., and KARSTEN, G., *Lehrbuch der Botanik für Hochschulen*. 16th ed. 8vo. pp. vi+685. Jena: Gustav Fischer. 1923.

³ STRASBURGER, E., and KOERNICKE, M., *Das Botanische Praktikum*. 7th ed. 8vo. pp. viii+883. Jena: Gustav Fischer. 1923.

⁴ MASSART, J., *Éléments de Biologie Générale et de Botanique*. 8vo. pp. ix+406 (2nd vol). Brussels: Lamertin. 1923.

family, genus, species. There will probably be more difference of opinion in regard to the subdivisions of the phylum VII than to any of the others. Under the Bryophyta there are two classes, the mosses and liverworts, with the mosses placed first, a sequence which will hardly meet the approval of morphologists. Under the Pteridophyta, Lycopodiaceae and Isoetaceae are classes of equal rank. Some of us would regard Lycopodiales as an order with Isoetaceae as a family under that order. Sphenophyllales is treated as an order under the class Equisetaceae. While I believe the two assemblages are closely related, I should prefer to treat them as coordinate orders. The "Cycadofilicinae" are placed under the Pteridophytes. This position is questioned, for the seeds are highly developed and the author himself recognizes gymnosperm characters. So long as gymnosperms are defined as they now are, even in WETTSTEIN's diagnosis, the Cycadofilicales should be regarded as an order of the gymnosperms.

The clear and interesting text, the extensive bibliographies, the splendid illustrations, and the careful work of a veteran botanist have produced a book which will be useful to morphologists, physiologists, and ecologists, and should suggest modern ideas of phylogeny to the professional taxonomist.

Das Botanische Praktikum made its appearance nearly forty years ago, and four editions were published during STRASBURGER's lifetime. In the fourth edition, much of the revising was done by KOERNICKE, who is responsible for all changes and improvements since that time. The present edition, the seventh, has been thoroughly revised and some portions have been entirely rewritten. Originally this book was intended to enable the student to teach himself the methods of preparing plant material for microscopic study. This object has been maintained, and microtechnique, as it has been developed at the Bonn school, is well presented, for KOERNICKE not only had the advantage of a long and intimate association with STRASBURGER, and with advanced students of many nationalities who came to study with the great botanist, but he has developed his own methods and has become a very independent technician.

In addition to directions for making slides and using all kinds of apparatus connected with microscopy, there are directions for obtaining material and studying it, so that the work is really a textbook of morphology and microchemistry, as well as a textbook of technique. Since there is still some difficulty in obtaining botanical literature, the author asks that papers involving technique and other features covered by the book be sent to him for use in future editions.

The "Bonn text," the first edition of which, by STRASBURGER, NOLL, SCHENCK, and SCHIMPER, appeared in 1894, has now reached its sixteenth edition. SCHENCK is the only one of the original authors now living. SCHIMPER was replaced by KARSTEN, who has written the special morphology of the seed plants, ever since the sixth edition appeared in 1903. NOLL wrote the physiology for the first nine editions, and at his death was succeeded by JOST. STRASBURGER, who planned the textbook and wrote the morphology of the first eleven

editions, died in 1912, since which time that part has been written by FITTING. Throughout the thirty years the general plan has been maintained, and through the numerous editions the authors have endeavored to keep the book up-to-date, rather than to introduce any radical changes.

It would seem better to combine whatever morphology there is in the first part with the morphology given in the third and fourth parts, and to amplify the ecological anatomy and physiology of the first part so as to make a section on ecology. As it stands, ecology has no adequate presentation, and alternation of generations and phylogeny, while receiving some mention, do not make such an impression as they would if presented in connection with the various groups. The physiological section, to one who is only a morphologist, is interesting and valuable, for it presents a modicum of physiology which every morphologist ought to know. On the whole, the book, with its 849 illustrations, is indispensable, and will continue to hold its high place.

MASSART's book is a general text, of college grade, covering morphology, physiology, ecology, paleobotany, and geographic botany. The reviewer has seen only the second volume, which begins with the Bryophytes, in which the *Anthoceros* assemblage is treated as a group coordinate with the Hepaticae and Musci. The mode of treatment of the various groups may be illustrated by the Pteridophytes and Phanerogams, the vegetative structures of which are treated together. There are two general headings, vegetative structures and reproductive structures. Under the first there are four topics, histology, the root, the stem, and the leaf. The root then, for example, is treated under the headings anatomy and external morphology, and anatomy and internal morphology. The internal morphology is then considered under primary structure, origin of primary structure, origin of the vegetative point, and secondary structures. Other features are treated similarly, and remarks on physiology and ecology are everywhere interspersed, although 157 pages are devoted to these fields in part IV. Under the heading "Origin of primary structure," while there is nothing new, the presentation is good and it is interesting to note that this subject is receiving attention.

Like WETTSTEIN, MASSART treats the Cycadofilicales under the Pteridophytes, as a group coordinate with the Filicales, Equisetales, and Lycopodiales. Unless the seed be abandoned as a basis of classification, the reviewer does not see how this group can be placed anywhere except under the gymnosperms. While I have no doubt that the Cycadofilicales developed from heterosporous Filicales, and that development was gradual, with the first seeds very small and doubtless hard to distinguish from large megaspores; nevertheless, when the seed condition was once attained the gymnosperm level was reached, and the fernlike seed plant should be ranked as a gymnosperm.

The book will be useful for reference, and it gives some idea of the general trend of botany in Belgium. The second volume is well illustrated, with 563 figures, many of which are new. Those which are copied are credited, but there is no bibliography.—C. J. CHAMBERLAIN.

A flora of the White Mountain region

Botanists have a professor of Greek to thank for one of the most substantial and scholarly local floras that has appeared for a long time. Dr. A. S. PEASE, formerly of the University of Illinois, now of Amherst College, became deeply interested in the plants of the White Mountains during his student days at Harvard. In 1907 he formulated a plan for the systematic study of the region, to culminate in the present publication.⁵ Before his investigations, interest in the botany of Coös County (an area of 1798 square miles, comprising the northern end of New Hampshire) had almost been confined to Mount Washington and the other mountains of the Presidential Range. With some assistance from other members of the New England Botanical Club, Professor PEASE has pursued a program of field work in Coös County every summer, except one, since 1906, gradually completing his records for the less frequented and previously unbotanized parts. By extending his work beyond the higher mountainous area, the author has been better able to trace the geographic relationships of the several components of the flora. For instance, he has traced various southern species up the valleys of the Connecticut, Androscoggin, and Saco rivers, the chief migration routes for southern types, and has located the barriers to their northward extension.

The title of the *Coös Flora* emphasizes that the author has not confined his attention strictly to the White Mountains. One wonders, however, how many botanists and geographers outside of New England identify the White Mountains with Coös County at all. The definiteness of the title may conceal the work from many who would surely find it valuable. Coös County includes all of the markedly alpine part of the White Mountains, and its flora is therefore of interest to everyone who is interested in phytogeography.

Before the publication of this *Flora* it could be said that no other part of the country as much visited by botanists was as imperfectly known. The fact that the height of interest in the White Mountain flora came before the development of modern critical and intensive methods resulted in a dearth of scholarly publication later on. There was doubtless a feeling that everything that could be said had been said somewhere, sometime, but as a matter of fact the literature was both inaccessible and inadequate.

The older publications were in some cases so inaccurate and careless that they were merely pitfalls for unwary compilers. No published records were accepted for the new flora without conscientious verification, either through searching out old herbarium specimens for critical identification or through rediscovery of the species in the field. The utmost that could be gleaned from the early literature was utilized, but only if the data could be verified. As a result, PEASE's *Flora* ranks with the best that have been produced. In some groups, as, for example, in *Crataegus*, additional species will doubtless be discovered, especially in the northern part of the county. Only in this one genus

⁵ PEASE, A. S., Vascular flora of Coös County, New Hampshire. Proc. Boston Soc. Nat. Hist. 37:39-388. pls. 5-II. 1924.

have the determinations (made at the Arnold Arboretum) been accepted by the author without critical study of his own. In other critical genera, as in *Rubus*, the author acknowledges aid from Professor FERNALD, and the whole work shows the influence of the Gray Herbarium. The authenticating specimens are mostly in the Herbarium of the New England Botanical Club (containing PEASE's own collections), the Gray Herbarium, and several other New England collections. In addition, the National Herbarium and the Herbarium of the New York Botanical Garden have been utilized.

An excellent phytogeographical and ecological discussion precedes the systematic list. Topography, soil, and climate receive sufficient consideration. The presentation of a twofold classification of habitats, separating those essentially natural from those modified by man, gives occasion for comments upon the previous ecological work in the region. The habitat lists are unusually full, and of course have an accuracy by no means usual.

There is a new summary of the broad relations of the alpine flora, in which it is shown that the endemic element is negligible, consisting only of four plants, a willow, *Salix Peasei*, which is probably a hybrid of *S. herbacea* and *S. Uva-Ursi*, and therefore may be expected in other arctic-alpine regions where the widely distributed parents occur, *Houstonia coerulea* var. *Faxonorum*, a variety of a wide spread lowland plant, and two well marked species, *Geum Peckii* and *Potentilla Robbinsiana*. If, following the history of the other supposed endemics, the latter species were yet to be discovered elsewhere, no ancient endemics whatever would remain of the long list that used to be quoted.

A bibliography, a list of botanical collectors chronologically arranged, and tables of distributional statistics close the introductory portion. The catalogue which follows gives detailed records of localities and exsiccatae for 1297 distinguishable species, varieties, and forms. There are a few well chosen plates illustrating some of the more interesting species. If the flora could have been expanded, additional illustrations of the same character and a series of plates illustrating typical habitats might well have been added. Perhaps there will be a second edition, or a series of more detailed specialized papers to come. As it stands, the *Flora of Coös County* is a model of painstaking local botany. All systematists will be grateful to the Boston Society of Natural History for undertaking its publication.—H. H. BARTLETT.

The plastids

A monograph giving a detailed account of the morphology, cytology, composition, physiological activities, and pathology of the plastids has been prepared by SCHÜRHOFF.⁶ The volume forms a part of the *Handbook of plant anatomy* now appearing in parts under the editorship of LINSBAUER. This summary of plastid organization and behavior will be useful to students of anatomy and physiology, and the long list of citations in itself will be a great

⁶ *Schürhoff, Die Plastiden. Ein Handbuch der Pflanzenanatomie.* Leipzig, 1923.

help to those who wish the original contributions. The work is mainly a compilation, as the author has not published any contributions to our knowledge of the plastids from his own investigations. In bringing together the literature, the European contributions have been well selected; but, as so frequently happens now, not enough attention has been given to the work done outside of European laboratories.

With reference to the last section of the volume, which deals with the pathology of the plastids, including abnormalities in form, chloroses, metaplastic greening, and mosaics, the discussion does not go far enough to be of any considerable help to those studying mosaics, but it gives a general idea of what has been done in Europe. It fails to reflect the present widespread investigation of mosaic diseases in America.

Although the volume has some quite obvious shortcomings, it brings together a great mass of scattered knowledge; and cytologists, anatomists, physiologists, and pathologists will all have occasion to study it, and it will form a valuable part of the completed handbook.—C. A. SHULL.

Handbook of British mosses

A third edition of DIXON'S *Handbook* has just been issued. It has given opportunity to include the additions to the British flora since 1904, the date of the second edition. The first edition was issued in 1896. The plates have been entirely redrawn and reproduced by photography, every figure in the book having been drawn from nature. The introduction contains an account of the structure of mosses, their classification, nomenclature, etc.

The book contains 712 species in 115 genera. The largest genus is *Hypnum* (56 species), followed by *Bryum* (45 species), *Grimmia* (34 species), and *Dicranum* (30 species). *Sphagnum* is represented by 18 species. This new and revised edition was certainly called for, and will prove to be a great help to students of mosses.—J. M. C.

MINOR NOTICES

Permeability

The papers on permeability by STILES, which ran through several volumes of the *New Phytologist*, and which were recently reviewed by DAVIS,⁸ have been reprinted in book form as *New Phytologist Reprint* no. 13.⁹ The list of more than 800 literature citations at the close of the book is in itself a valuable contribution to plant physiology. The English authors and publishers are to be congratulated upon their ability and willingness to bring out such summaries of the progress of research in special fields.—C. A. SHULL.

⁷ DIXON, H. N., *The student's handbook of British mosses*. 3d. ed. xlviii+582. pls. 63. London: Weldon and Wesley. 1924.

⁸ BOT. GAZ. 78:245. 1924.

⁹ STILES, WALTER, *Permeability*. 8vo. pp. viii+296. London: Wheldon & Wesley, Ltd. 1924.

NOTES FOR STUDENTS

The hydrogen ion.—MISS BROOKS¹⁰ finds *Bacillus butyricus* and *B. subtilis* very sensitive to the reaction of the growth medium. *B. butyricus* produces carbon dioxide most rapidly at P_H 7, *B. subtilis* at P_H 6.8. As the growth medium is made more acid or alkaline, there is a progressive reduction in the amount of carbon dioxide produced. Spontaneous recovery follows the addition of alkali to the growth medium. Alkali must be added to cause recovery after the addition of acid, and then the recovery is not complete unless little acid was added.

Working under JONES at Wisconsin, SHERWOOD¹¹ has studied the relation of the soil reaction to the development of the *Fusarium* wilt of the tomato. The percentage of diseased plants was greatest in the more acid soils. The critical time of infection seemed to be within the first three days following germination. The experiment indicated that the fungus is able to grow within the range of hydrogen ion concentrations likely to occur in the soil. A P_H of 2 seemed to be required to prevent spore germination. These two papers again illustrate the sensitivity of the lower organisms to the reaction of the growth.—S. V. EATON.

Hepaticae of Switzerland.—MEYLAN¹² has published a very full and admirable account of the liverworts of Switzerland. The prefatory pages include a history of research in the group, a list of publications dealing with Swiss material, an account of the organography and its terminology, the biology of the group, the variability and value of species, the ecological associations, and the liverwort regions of Switzerland.

The taxonomic presentation furnishes a good illustration of the relative abundance of the liverwort groups. The author recognizes in Switzerland 69 genera and 260 species. Of these the Jungermanniales include 52 genera and 223 species; the Marchantiales 16 genera and 22 species; the Anthocerotales a single genus with 5 species. By far the largest genera are *Lophozia* (28 species), *Scapania* (26 species), and *Cephalosziella* (18 species). It is interesting to note that only a single new genus (*Cladopus*) and species (in *Grimaldia*) are proposed.—J. M. C.

Distribution of marine algae.—A recent contribution by SVEDELIUS¹³ is particularly welcome on account of the scanty literature dealing with either

¹⁰ BROOKS, MATILDA M., The effect of hydrogen ion concentration on the production of carbon dioxide by *Bacillus butyricus* and *B. subtilis*. Jour. Gen. Physiol. 4:177-186. figs. 3. 1921.

¹¹ SHERWOOD, E. C., Hydrogen ion concentration as related to the *Fusarium* wilt of the tomato. Amer. Jour. Bot. 10:537-552. 1923.

¹² MEYLAN, CH., Les Hepatiques de la Suisse. Beitrage Kupt. der Schweiz. 6:1-318. figs. 213. 1924.

¹³ SVEDELIUS, NILS, On the discontinuous geographical distribution of some tropical and subtropical marine algae. Arkiv. för Botanik 19: pp. 70. 1924.

the ecological or floristic distribution of marine algae. In his introduction, he comments on the unsatisfactory character of our present knowledge, and on the futility of attempting to solve the problems of the discontinuous distribution of various forms on the basis of existing conditions. He cites his explanation, given in 1905, of the similarity of the West Indian and Indo-Pacific floras based on the evidence that the Pacific Ocean and the Caribbean Sea were formerly continuous. He then gives details, illustrated by maps, of the distribution of various families and genera which support the hypothesis. Similar evidence supports the hypothesis that the Mediterranean has received several forms from the south during Tertiary times, when an open connection with the Indian Ocean existed.—GEO. D. FULLER.

Vegetation of Canada.—There has recently appeared a decidedly good map of the principal plant formations of Canada.¹⁴ The ecological viewpoint is taken and the mapping seems to be very accurate. The forest types recognized are the northern hygrophytic evergreen, the northern conifer, the northern mixed, the eastern conifer, the hardwood, the mixed, and the Carolinian. In addition, the transitional grove belt, the prairie grassland, and the cleared portion of the mixed forest belt are delimited. It would be an improvement if an attempt had been made to confine the map entirely to the native vegetation, although the area cleared and farmed is principally from the mixed hardwood-conifer and Carolinian formations. This latter area is limited to the portion of Ontario south of the Georgian Bay possessing originally a forest cover similar to that of Ohio and western New York.—GEO. D. FULLER.

Translocation.—By means of girdling and defoliation experiments, CURTIS¹⁵ has offered excellent evidence that there is inadequate longitudinal translocation of foods by the xylem of woody plants, and that the longitudinal movement of the foods is mainly in the phloem. "Although large amounts of carbohydrates are stored in xylem tissues, there is no appreciable longitudinal transfer of sugars through these tissues."—WM. CROCKER.

Cyanogenesis of Sudan grass.—MENAU and DOWELL¹⁶ find that Sudan grass contains about one-third as much hydrocyanic acid as the grain sorghums. The quantity is greatest in the young plant, and decreases rapidly as the plant matures.—WM. CROCKER.

¹⁴ LYNCH, F. C. C., Map of the southern portion of the Dominion of Canada indicating vegetation and forest cover. 20×35 inches. Dept. of Interior. Ottawa, Canada. 1924.

¹⁵ CURTIS, O. F., The upward translocation of foods in woody plants. I. Tissues concerned in translocation. Amer. Jour. Bot. 7:101-124. 1920.

¹⁶ MENAU, P. and DOWELL, C. T., Cyanogenesis of Sudan grass: a modification of the Francis-Connell method of determining hydrocyanic acid. Jour. Agric. Res. 18:447-450. 1920.

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Classified entries will be found under Contributors and Reviewers. New names and names of new genera, species, and varieties are printed in **bold-face** type; synonyms in *italic*.

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